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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A01N 25/02, 25/08, A61F 2/04
A61M 25/01

A1

(11) International Publication Number: **WO 94/10838**

(43) International Publication Date: 26 May 1994 (26.05.94)

(21) International Application Number: PCT/US93/10893

(22) International Filing Date: 12 November 1993 (12.11.93)

(30) Priority data:
07/975,486 12 November 1992 (12.11.92) US

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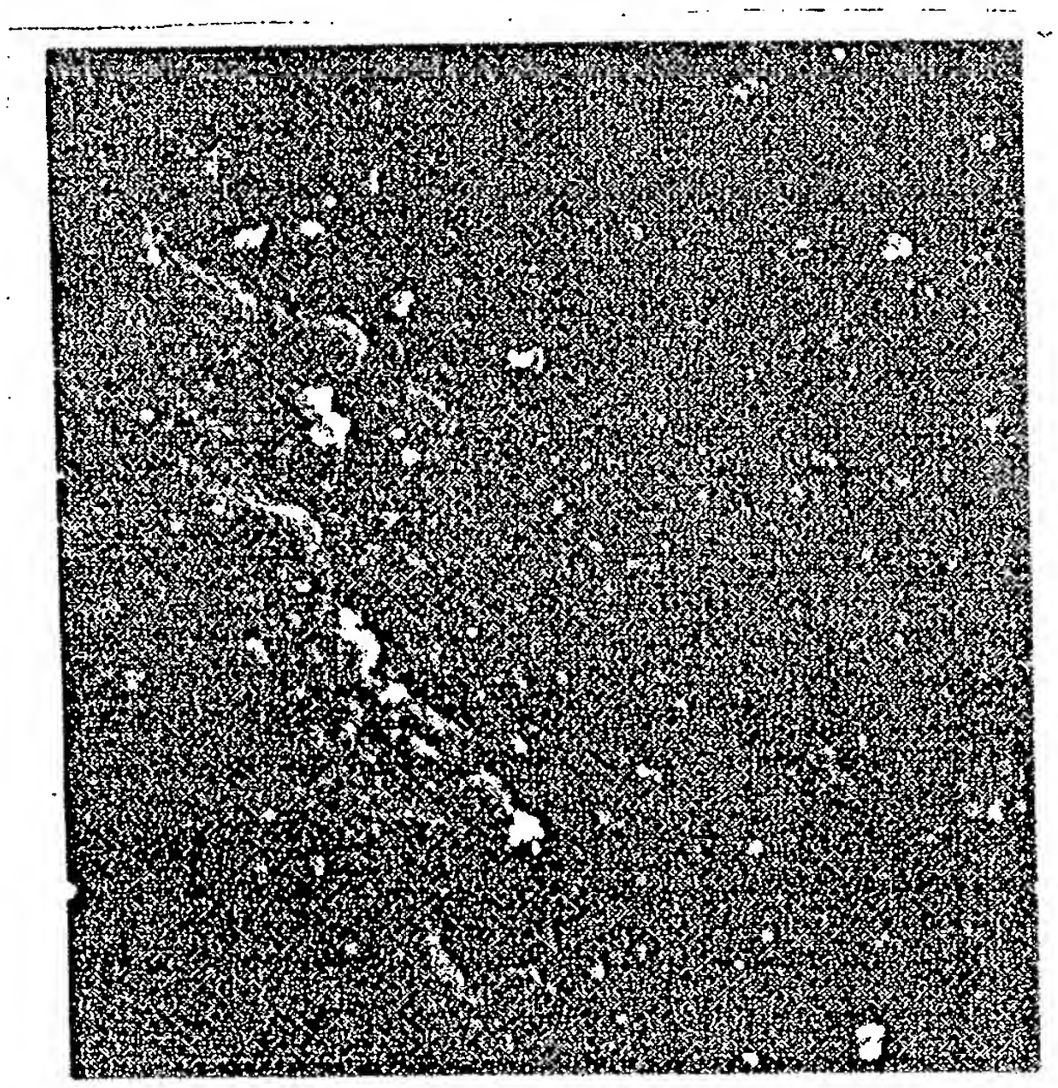
(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: M-EDTA PHARMACEUTICAL PREPARATIONS AND USES THEREOF

(57) Abstract

Disclosed are pharmaceutical compositions of a mixture of minocycline and EDTA (M-EDTA) and methods of using the compositions in maintaining the patency of a catheter port. Methods for inhibiting the formation of polysaccharide-rich glycocalyx (such as the glycocalyx of staphylococcal organisms) are also provided using an M-EDTA solution. A solution including minocycline, EDTA or both, may also be used to pretreat a medical device to prevent adherence of infectious organisms, such as *S. epidermidis* and *S. aureus*. The compositions destroy and prevent the formation of polysaccharide-rich glycocalyx. Methods for treating infections of *S. epidermidis* and *S. aureus* where glycocalyx formation are provided with an M-EDTA solution. The minocycline and EDTA solutions are included together within a pharmacologically acceptable carrier solution, such as saline.



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DESCRIPTION

M-EDTA PHARMACEUTICAL PREPARATIONS AND USES THEREOF

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

The present invention relates to indwelling medical articles, such as catheters, which may also be flushed or coated with a microbial-inhibiting pharmaceutical preparation. The invention also relates to pharmaceutical preparations useful in maintaining catheter patency and preventing infection. Methods of using the pharmaceutical preparation of the invention in the management and maintenance of a vascular catheter are also related to the present disclosure.

2. BACKGROUND OF THE RELATED ART

Indwelling medical devices including vascular catheters have become essential in the management of hospitalized or chronically ill patients. Unfortunately, vascular catheters have become the major source for hospital-acquired sepsis. Hence, the benefit derived from indwelling medical devices such as vascular catheters is often upset by infectious complications. Thrombotic occlusions of the lumen of central venous catheters (CVC) is another complication that will often lead to the removal of catheters.

The current standard care of catheters includes flushing the lumen of the catheter with heparin. However, heparin has no antimicrobial activity. Thus, infections, as well as thrombotic occlusion, continue to occur frequently despite the prophylactic use of heparin flushes. Knowledge of the pathogenesis and microbiology

of central venous catheter-related infections is essential in order to provide effective prevention for this problem. Three essential factors must be considered in controlling for catheter colonization by infectious microbes. The first is controlling the availability of microorganisms that adhere to the inert catheter surface. Such microorganisms typically include staphylococci and candida. The second is controlling the production of a polysaccharide by adherent known as organisms fibrous glycocalyx. Production of the glycocalyx is essential for the adherence and integrity of these organisms. The third is control of the formation of a thrombin sheath by the host, which acts to engulf the catheter. The thrombin sheath provides the microorganisms a sticky substrate for enhanced adherence to the catheter, and thus, continued colonization and infection at the catheter site. The present inventors herein disclose an M-EDTA solution unique in its ability to inhibit all three of these essential conditions, and thus provide effective methods for controlling catheter-related infection and onset thereof.

Staphylococcus epidermidis and *S. aureus* account for 75% of CVC related infections. *Candida* species account for another 10% to 15% of such infections. The use of antistaphylococcal antibiotics to prevent these infections has been found to reduce CVC related bacterial infections, but only at the expense of the occurrence of higher rates of fungal (*Candida*) infections. The fibrous glycocalyx material produced by staphylococci and Candida helps these organisms adhere and stick to catheter surfaces, thus exacerbating the problem of eliminating these types of infections after they have become established. These microbial biofilm layers are made of a fibrous glycocalyx material primarily polysaccharide in nature. The protective sheath provided by the glycocalyx at the infected site effectively prevents the elimination

and treatment of these infections. Preparations effective for destroying such a glycocalyx would, therefore, provide a solution for treating established catheter infections, particularly where a glycocalyx is already formed.

Compositionally distinct glycocalyx material is produced by a variety of different organisms. For example, a mostly protein glycocalyx produced by *Hymenolepis diminuta* (tapeworm) is reportedly eliminated upon treatment with 0.02 M-EDTA or 3 M KCl[12]. This material, however, is compositionally distinct from the material of the glycocalyx formed by organisms that typically colonize and cause catheter infection. For example, the glycocalyx of several staphylococcus species comprise primarily polysaccharides with only low to nondeductible levels of protein[13] (Tojo *et al*. at pg. 716, Table 1). Glycocalyx of microorganisms common to catheter infection are thus compositionally distinct from the glycocalyx of such organisms as the tapeworm, *Hymenolepis diminuta*. A pharmaceutical preparation effective for reducing or eliminating glycocalyx of infectious microorganisms typically associated with catheter colonization and infection has yet to be identified.

Infectious microorganisms will typically embed themselves in the protective layer of the glycocalyx, thus providing a shield or hiding place that protects staphylococci and fungi from the activity of the host's phagocytic cells. An agent or composition that would dissolve or prevent the glycocalyx (biofilm) formation of these clinically important pathogens would thus provide a major breakthrough in the prevention of typical catheter-related *Staphylococcal* and *Candida* infections.

There has also been observed to be a correlation between thrombogenesis and infection. Indwelling vascular catheters are typically engulfed by a fibrin sheath that subsequently acts to cover the internal and external surfaces of a catheter. This fibrin sheath provides such organisms as *Staphylococci* and *Candida*, with an enhanced adherence capacity to the catheter surface. Unlike these particular microbes, gram-negative bacilli do not adhere well to fibrin and fibronectin. A composition that halts fibrin formation would thus be particularly useful in halting the colonization of these microbes at indwelling catheter sites.

Intraluminal colonization through a catheter hub also constitutes a prelude to catheter-related infections and septicemias in long-term CVC. The inventors study presented herein of long-term CVC patients (studied by quantitative catheter culture) demonstrates that CVC's with positive cultures as well as matched negative controls evidenced colonization (as quantitated by EM) and biofilm formation of the internal surface at least twice greater than that of external surface with catheters that stayed longer than 10 days in place. This data is from nontunneled, noncuffed percutaneous CVC. For tunneled CVCs (Hickman/Broviac) and ports, internal colonization was even more prominent. The development of an anticoagulant pharmaceutical preparation effective against staphylococci, fungi, and polysaccharide-rich glycocalyx formation, such as that observed during microorganism intraluminal colonization of a catheter hub, would provide a solution to the treatment and elimination of thrombogenesis and the septicemia associated with long-term CVC.

EDTA is an anticoagulant used in blood collection, and is also described as having an antibacterial and

antistaphylococcal effect (alone or in combination)[1-3]. Root[9] described the efficacy of EDTA in vascular catheters as an antibacterial agent as compared to heparin alone and as compared to a vancomycin-heparin preparation *in vitro*. These investigators found EDTA to have some bacteriocidal activity. However, no remedy or suggestion for treatment of how a microbial glycocalyx, such as that observed during *in vivo* catheter-related infection, was observed or described.

Glycopeptide antibiotics (vancomycin and teicoplanin) are active against staphylococci *in vitro* and in tissue. Vancomycin is currently the standard antibiotic used in the treatment of *Staphylococcus epidermidis* and resistant *Staphylococcus aureus*. However, these antibiotics are not active against adherent staphylococci embedded in a biofilm layer, such as glycocalyx. In addition, while catheter flushing with such agents may destroy initial strains of staphylococci, such is typically not effective against tolerant and resistant strains of the organism that continue the infection. Flushing with an antibiotic preparation, such as vacomycin, would therefore be of only limited therapeutic value against catheter infection.

The ideal prophylactic agent or treatment for catheter maintenance would inhibit or eliminate the formation of polysaccharide-rich glycocalyx of microorganisms and would also inhibit staphylococci and fungi infectious growth.

It is an object of the invention to provide both an anti-staphylococcal and antifungal (anti-*Candida*) active agent effective against free-floating as well as adherent organisms embedded in biofilm, as well as to provide an anticoagulant agent and/or method that would prevent or inhibit the formation of polysaccharide-rich fibrous

glycocalyx biofilm layer. Such a pharmaceutical agent would optimally prevent thrombotic occlusion of the catheter lumen and prevent thrombin formation. Additional objects of the invention include providing an agent that could be given intraluminally without a toxicity concern to humans and to provide a method that would kill adherent *Staphylococci* and *Candida*. Such methods would preferably not include the use of pharmaceutical agents typically used to treat non-catheter related infections (such as Vancomycin, Ampho B, or Azoles).

The present invention demonstrates that a mixture of minocycline/disodium EDTA (referred to as M-EDTA) does fulfill all of the listed objects.

SUMMARY OF THE INVENTION

The present invention provides a unique and effective pharmaceutical preparation that includes minocycline and EDTA. These mixtures are shown to be effective for maintaining the patency of a medical device, such as a catheter, *in vivo*. As used in the description of the present invention, "patency" is defined as the state of being freely open or patulous, particularly a catheter opening that is unobscured by the formation of a microorganismal polysaccharide-rich fibrous glycocalyx. In a preferred embodiment, minocycline and EDTA are included in the disclosed preparations in pharmacologically effective amounts together in a pharmacologically acceptable carrier solution.

The EDTA of the preparation provides potent glycocalyx inhibiting potential, while the minocycline at high concentrations has a fungicidal effect and a unique ability to penetrate a polysaccharide-rich glycocalyx

biofilm layer. The combination of minocycline-EDTA thus provides a uniquely effective anticoagulant, anti-microbial, glycocalyx inhibiting, antibacterial and antifungal agent for the prevention of thrombogenesis, microbial adherence and device-related infections.

The inventors demonstrate that minocycline has the surprising activity of penetrating a polysaccharide-rich microbial glycocalyx biofilm layer at least 6-fold more effectively than vancomycin. The inventors also demonstrate that EDTA effectively prevents and dissolves polysaccharide-rich microbial glycocalyx formation at implanted catheter sites *in vivo*.

In still another aspect of the invention, methods for using minocycline as an antimicrobial agent are provided. Minocycline is demonstrated to kill adherent staphylococci (embedded in glycocalyx - Example 4), and to be superior to vancomycin as an antibacterial agent (Table 3) in actual *in vivo* trials.

For use in maintaining catheter patency, the pharmaceutical preparation of the invention may be efficaciously used in conjunction with virtually any device in which a clear path for the flow of biological fluids is necessary. For example, such devices include a central venous catheter, a peripheral intervenous catheter, an arterial catheter, a Swan-Ganz catheter, a hemodialysis catheter, an umbilical catheter, a percutaneous nontunneled silicone catheter, a cuffed tunneled central venous catheter as well as a subcutaneous central venous port.

In a preferred embodiment, a solution of the M-EDTA may be prepared containing a concentration of between about 10 to about 100 mg/ml EDTA (preferably between about 20 to about 60 mg/ml) and between about 0.001 to

about 100 mg/ml minocycline (preferably between about 2 and about 9 mg/ml). Most preferably, the preparation as a device or catheter flushing solution includes about 30 mg/ml EDTA and about 3 mg/ml minocycline. The minocycline may preferably be prepared as a reconstituted minocycline preparation, such as from a 100 mg vial of minocycline commercially available (Minocin Intravenous, Lederle (Carolina, Puerto Rico). The carrier solution, by way of example, may comprise saline, preferably sterile saline, available from commercial sources.

In another aspect of the invention, a catheter flushing solution is provided. Most preferably, the catheter flushing solution comprises a glycocalyx inhibiting concentration of EDTA and minocycline in a pharmaceutically acceptable carrier solution. More specifically, the concentration of EDTA in the flushing preparation is between about 1 to about 100 mg/ml with the concentration of minocycline being between about 0.001 to about 100 mg/ml in the preparation. Most preferably, the catheter flushing solution includes about 30 mg/ml EDTA and about 3 mg/ml minocycline.

By way of example, the carrier solution of the flushing preparation is saline, preferably sterile saline. The catheter flushing preparation of the present invention may advantageously be used to inhibit the formation of polysaccharide-rich glycocalyx. In this manner, infections characterized by the presence of a polysaccharide-rich glycocalyx, such as a glycocalyx which is at least 50% polysaccharide by composition, may be effectively treated and/or eliminated.

A "glycocalyx inhibiting concentration" is defined for purposes of describing the present invention as a concentration of minocycline, EDTA or a combination thereof effective to degrade, dissolve, or otherwise

inhibit polysaccharide-rich glycocalyx formation. By way of example, a polysaccharide-rich glycocalyx is characteristic of established staphylococcal infections of *S. aureus* and *S. epidermidis*.

Another aspect of the present invention provides a method of preparing a biofilm-resistant device, as well as bio-film resistant devices that are coated with at least one of minocycline or EDTA. Most preferably the device is to be coated with a mixture of minocycline and EDTA. The method in one embodiment comprises coating a device with a coating preparation minocycline and EDTA. While the method may be used to coat virtually any surface where glycocalyx formation is to be desirably inhibited, use of the method in coating a catheter device is particularly envisioned. It is anticipated that the method will provide a device resistant to polysaccharide-rich (at least about 50% polysaccharide) glycocalyx formation, such as that characteristic of Staphylococci. Any of a variety of catheters may be treated or coated according to the described method employing coating techniques well known to those of skill in the art. By way of example, catheters that may be prepared and treated according to the invention include a central venous catheter and a triple lumen catheter.

In a most preferred embodiment, the method for preparing a biofilm-resistant medical device using a pharmaceutical preparation of minocycline and EDTA comprises preparing a solution of at least one of minocycline or sodium EDTA, most preferably in a biocompatible adherent coating carrier solution, and treating the surface of the medical device of interest with the solution for a period of time sufficient to allow the formation of a film of the minocycline, EDTA, or both to the surface of the device. Most preferably,

the method is to be used in preparing a biofilm-resistant coating on the surface of a catheter.

Alternatively, a coating of the M-EDTA preparation may be provided at the surface of a device by first treating the surface of the device with a surfactant. Such surfactants include, by way of example, tridodecyl-methyl ammonium chloride (TDMACC). Commercially available catheters with such a surfactant already included on the surface are the Bioguard Cook catheters. Accordingly, a device including a surface surfactant may be immersed in a solution of minocycline, EDTA or M-EDTA for a period of time sufficient to allow the solution to permeate the device surface. By way of example, the present inventors have found that immersion of a device in a solution containing about 60 mg minocycline and/or about 60 mg EDTA/ml for about 15 minutes at room temperature is sufficient to provide a coating of the solution to the surface of the device. The device is preferably air dried and gas sterilized prior to use.

As used in the description of the present invention, a "biofilm-resistant" device or surface is defined as a surface or device that will prevent the adherence or growth of organisms that produce polysaccharide-rich (about 50% or greater polysaccharide) formations, such as those formations generally referred to as a glycocalyx. Organisms that produce such a glycocalyx include the Staphylococcal organisms, particularly the S. aureus and S. epidermidis species. However, any organism that produces a polysaccharide-rich material would be equally inhibited by the herein described devices, surfaces, pharmaceutical preparations and methods.

The present methods are particularly efficacious for inhibiting polysaccharide-rich glycocalyx formation at a catheter port. The method in one embodiment comprises

flushing the catheter periodically with a preparation including a glycocalyx-inhibiting concentration of at least one of EDTA and minocycline, pharmacologically acceptable carrier solution. In one aspect of the method, the glycocalyx-inhibiting concentration of EDTA in the flushing solution is between about 0.01 mg/ml and about 100 mg/ml. The most preferred concentration of EDTA is about 30 mg/ml. According to one aspect of the described method, the catheter may be described as a tunneled catheter or an untunneled catheter. As part of a catheter maintenance regimen, the catheter most preferably is to be flushed with the aforedescribed preparation at least once every about 24 to about 48 hours.

In still another aspect of the method, the pharmaceutical preparation includes minocycline. Where included, the glycocalyx-inhibiting concentration of minocycline constitutes a concentration of between about 0.001 mg/ml and about 100 mg/ml. The most preferred concentration of minocycline is about 3 mg/ml.

Particularly stated, the method for eliminating microbial glycocalyx formation, particularly polysaccharide-rich (Staphylococcal) glycocalyx formation, at a catheter lumen comprises preparing a solution comprising minocycline and EDTA in a carrier solution to provide an M-EDTA preparation, and flushing the catheter with a therapeutically effective amount of the M-EDTA preparation sufficient to reduce a polysaccharide glycocalyx. In one embodiment, the M-EDTA preparation includes a concentration of minocycline of between about 0.001 to about 100 mg/ml (preferably between about 2 and about 9 mg/ml) and between about 10 to about 100 mg/ml (preferably between about 20 to about 60 mg/ml) EDTA. The therapeutically effective amount of the aforedescribed M-EDTA preparation would, therefore,

constitute between about 1 to about 10 ml (preferably about 2 to about 3 ml) of the aforedescribed solution.

According to the method, a volume of about 3 ml of the aforedescribed M-EDTA preparation containing about 30 mg/ml EDTA and about 3 mg/ml minocycline is used per flush of a catheter or device, depending on the voume of the catheter or device being monitored. For example, a standard size tunneled CVC catheter (Hickman/Brovia) is to be flushed periodically at least once every about 24 hours to about 48 hours with between about 2 to about 3 ml of the M-EDTA preparation. In a preferred aspect of the method, the catheter is to be flushed more frequently, such as at least once every about 4 hours with the herein described preparations of M-EDTA.

The M-EDTA preparation will remain therapeutically effective for use as a catheter-flushing agent stored at a refrigerated temperature for at least 1 month after formulation. In use, the M-EDTA solution should be brought to room temperature before use on an animal or patient.

The aforedescribed preparations have been found effective in preventing the adherence and colonization of catheter surfaces by *S. aureus*, *S. epidermidis*, and fungi, as well as effective in both treating and eliminating already formed glycocalyx formations of these infectious organisms.

The anti-microbial resistant medical devices of the present invention are further described as a medical device that is processed to include a coating of minocycline, of EDTA or of a combination of M-EDTA. Such a coating would be particularly efficacious for medical devices that include at least one opening or portal, as the coating is shown by the present inventors to be

effective for maintaining the patency of such medical device openings as that characteristic of an indwelling catheter *in vivo*. The most preferred embodiments of the devices include a coating of M-EDTA. Coating solutions of the M-EDTA include about 60 mg minocycline and about 60 mg EDTA per ml.

The following abbreviations are used in the description of the present invention:

CVC	=	Central Venous Catheters
MRD	=	Modified Robbins Device
M-EDTA	=	minocycline-EDTA mixture
D_{10}/W	=	10% Dextrose and Water

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 - Scanning electron microscopy picture showing staphylococci and biofilm from a control catheter segment that was exposed to slime producing *S. epidermidis* and later immersed in Dextrinase for 24 hours. (Example 6 results.)

Figure 2 - Scanning electron microscopy picture showing a layer of biofilm from a catheter segment that exposed to slime producing *S. epidermidis* and later immersed in Urokinase for 24 hours. (Example 6 results.)

Figure 3 - Scanning electron microscopy picture showing a representative clear surface from a catheter segment exposed to slime producing *S. epidermidis* and later immersed in EDTA for 24 hours. The white particles are dust particles. (Example 6 results.)

Figure 4 - A scanning electron micrograph at high magnification showing coccal forms in a biofilm layer from a catheter segment coated with control solution

(saline) and later exposed to slime producing *S. epidermidis*. (Example 7 results.)

Figure 5 - Lower magnification from a different area of the catheter of Figure 4 showing coccal forms and biofilm. (Example 7 results.)

Figure 6 - Micrograph of a catheter surface pretreated with dextrinase. A scanning electron micrograph picture showing a catheter surface pretreated or coated with dextrinase, upon exposure to staphylococci. The micrograph shows a thick biofilm layer with many coccal formations. These coccal formations are indicative of staphylococcal colonization.

Figure 7 - Electron micrograph demonstrates formation of fibrous glycocalyx on the surface of a control (saline-treated) catheter segment - before flushing with saline for 4 hours.

Figure 8 - Electron micrograph demonstrates presence of deranged fibrous glycocalyx on the surface of a minocycline coated catheter segment - before flushing with saline for 4 hours.

Figure 9 - Electron micrograph demonstrates fibrous glycocalyx on the surface of a control (saline-treated) catheter segment - after flushing with saline for 4 hours.

Figure 10 - Electron micrograph demonstrating fibrous glycocalyx on the surface of a minocycline-coated catheter segment - after flushing with saline for 4 hours.

Figure 11 - EDTA Stability (A) and Minocycline Stability (B).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides pharmaceutically effective formulations of minocycline, EDTA and combinations thereof. These formulations have been found by the present inventors to be particularly useful in preventing the formation of the "biofilm" or polysaccharide-rich glycocalyx that typically accompanies microbial surface colonization. In particular, the formulations are most effective in breaking down polysaccharide-rich glycocalyx formations and in inhibiting their formation. This feature makes the preparations of the present invention potentially useful in the treatment of staphylococcal infections where a polysaccharide-rich glycocalyx has formed or may potentially be formed, as well as in the prevention and treatment of *Staphylococcal* and *Candida* infection.

The present invention also provides M-EDTA-treated or coated medical devices, such as catheters, that prevent staphylococcal or fungal colonization.

The minocycline (M) used in the studies described in the present disclosure was obtained from Lederle (Minocin® (intravenous, 100 mg, Carolina, Puerto Rico). The disodium-EDTA used in the studies described in the present disclosure was obtained from Abbott Co. (Endrate® (Intravenous 150 mg/ml) Chicago, IL). A Modified Robbin's Device, a screening tool customarily used and accepted as predictive of catheter use in humans[15,16], was used in the present study of the M-EDTA pharmaceutical preparations described. The model was constructed at M.D. Anderson Cancer Center in Houston, Texas.

The following agents were used in the studies disclosed herein:

SUBSTANCE	BRAND NAME	SOURCE	LOCATION
Urokinase	Abbokinase	Abbott Laboratories	Chicago, IL
Heparin	--	Sigma Chemical Co.	St. Louis, MO
Saline	0.09 Sodium Chloride (injection U.S.P.)	Baxter Healthcare Corp.	Deerfield, IL
Dextrinase	--	Sigma Chemical Co.	St. Louis, MO
Vancomycin	Lyphocin Intravenous, 1 gram	Lyphomed	Rosemont, IL
Trypticase Soy broth	--	DIFCO Laboratories	Detroit, MI

Even though the invention has been described with a certain degree of particularity, it is evident that many alternatives, modifications, and variations will be apparent to those skilled in the art in light of the present disclosure. Accordingly, it is intended that all such alternatives, modifications, and variations which fall within the spirit and the scope of the invention be embraced by the defined claims.

The following examples are presented to demonstrate preferred embodiments of the invention, but should not be construed as limiting the claims thereof.

It should be appreciated by those of skill in the art that the technique disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiment which are disclosed and still obtain like or similar results without departing, again, from the spirit and scope of the present invention.

EXAMPLE 1 - PREPARATION OF M-EDTA PHARMACEUTICAL PREPARATION

The present example provides a detailed description of how the M-EDTA pharmaceutical preparation was prepared for use as a catheter or medical device flushing solution. EDTA was obtained from Sigma. Minocycline was obtained from Lederle.

The M-EDTA solution was prepared as follows so as to achieve a concentration of about 3 mg/ml minocycline and

about 30 mg/ml EDTA in a sterile saline solution. Separate solutions of EDTA (60 mg/ml) and minocycline (3 mg/ml) were prepared in saline. The EDTA was reconstituted from 200 mg/ml Edetate Calcium Disodium (Versenste®, 3M Riker, Northridge, CA) or reconstituted from Edetate Disodium (150 mg/ml parenteral concentrate (Endtrate®, Abbott, Chicago, IL, or Disotate®, Forest, Maryland Heights, MO). Alternatively, the 60 mg/ml of EDTA could be reconstituted from EDTA powder (Sigma Chemical Co., St. Louis, MO). Minocycline was obtained from Lederle and combined with a volume of saline sufficient to constitute about 3 mg/ml minocycline.

The 6 mg/ml minocycline and 60 mg/ml EDTA solutions were mixed in equal volumes to constitute a 3 mg minocycline and 30 mg EDTA/ml solution. The solution was then brought to a physiologically acceptable pH of about 7.4. The solution was stored in a sterile container.

Once formulated, the M-EDTA may be stored refrigerated at 4°C until use. It is contemplated that so formulated, the solution will remain chemically stable and pharmacologically active for at least 1 month at 4°C. The preparation is also very stable at room temperature (37°C) for at least 72 hours (Table 1 and Figure 11). The preparation should be at room temperature before administration to a patient or animal.

Table 1. Percentage of Initial Concentration Remaining* of Calcium Disodium Versenate (EDTA) 30.0 mg/mL and Minocycline Hydrochloride 3 mg/mL in a Catheter Flush Solution. Detected by High Power Liquid Chromatography.

	Storage time (hr)					
Temperature (C)	0	6	24	72	120	168
EDTA						
37	100.0±0.5	99.9±0.6	100.3±0.2	97.9±0.4	99.0±0.5	96.9±0.2
25	100.0±0.5	102.1±0.3	100.9±0.0	100.1±0.1	100.4±0.2	100.5±0.2
4	100.0±0.5	100.2±0.1	100.8±0.2	101.7±0.8	100.3±0.1	100.9±0.4
Minocycline Hydrochloride						
37	100.0±0.2	99.76±0.0	96.0±1.3	79.9±0.7	65.4±1.0	53.5±1.3
25	100.0±0.2	100.5±1.9	99.4±0.8	98.7±0.5	96.8±0.5	93.8±0.6
4	100.0±0.2	98.9±0.4	99.7±0.5	98.8±0.7	98.3±0.6	99.8±0.1

* Mean of duplicate determinations ± S.E.M.

EXAMPLE 2

THE MODIFIED ROBBINS DEVICE MODEL

The present example is provided to describe the study model employed for illustrating the antimicrobial and therapeutic utility of the minocycline and EDTA preparations of the present invention.

An *in vitro* model, the Modified Robbin's Device (MRD), was used to study the formation of biofilm and colonization of catheter segments of *S. epidermidis*. This is a well established model that is described in Nickel *et al.*[15] and Evans and Holmes[16], and provides a study model recognized by those of skill in the art as predictive of *in vivo* effects at a catheter surface.

The MRD is constructed of an acrylic block, 42 cm long, with a lumen of 2x10 mm. The MRD is made of twenty-five evenly spaced specimen plugs each connected to a catheter latex segment whose anterior surface (0.3 cm^2) comes in contact with the flushed infusate coming from a connected tubing and infusion bag. Several studies were conducted using this model, which are outlined in the following examples.

EXAMPLE 3 - INHIBITION OF *S. EPIDERMIDIS*

The present example is provided to demonstrate the utility of the present invention for inhibiting *S. epidermidis* in and on a medical device, such as a catheter. The model described in Example 2 was used in the present study. These results demonstrate the utility of the invention for treating and maintaining catheter patency *in vivo*, and more specifically for inhibiting *S.*

epidermidis adhesion and glycocalyx formation at a catheter surface.

Catheter segments were placed in the specimen plugs of the Modified Robbins Device described in Example 2. After placing the catheter segments in the specimen plugs, the entire apparatus was sterilized with ethylene oxide. A 500 ml 10% dextrose/water bag was infected with $4x10^8$ CFU/ml of *S. epidermidis* (to produce $8x10^5$ CFU per ml of D_{10}/W). The infected infusate was flushed through the MRD for 3 hours at a 50 ml/hr (using a peristaltic pump). In order to remove all free floating and loosely adherent staphylococci, the infected bag was removed and a new sterile bag (of D_{10}/W) was used to flush the MRD. The MRD was flushed with sterile D_{10}/W for 24 hours at 40 mls/hr. Following this, catheter segments of equal size were treated with different agents by placing them in tubes containing one of the following solutions:

1. Urokinase (5000 units/ml);
2. Heparin (1000 unit/ml);
3. EDTA (50 mg/ml); and
4. Trypsin (20,000 units/ml).

Representative catheter segments were then removed (in a sterile manner) at 4 and 24 hours and quantitatively cultured using the scrape-sonication technique described by Khoury *et al.* (1991)[14] to isolate organisms adherent to catheter surfaces. The Khoury *et al.* reference is specifically incorporated herein by reference for this purpose. The experiment was done at 37°C.

The results from this study are presented at Table 2. The results demonstrate that treatment of catheter surfaces with EDTA was effective in preventing

adherent *S. epidermidis* colonies on a catheter surface after only 4 hours of treatment. In contrast, urokinase, heparin and trypsin treatment of the catheter segments was significantly less effective at inhibiting adherent *S. epidermidis* colony formation and adherence after 4 hours of treatment.

TABLE 2

NO. OF ADHERENT *S. EPIDERMIDIS* COLONIES OBTAINED FROM 0.3 CM^2 CATHETER SURFACES

Agent Used	After 4 hrs. of treatment	After 24 hrs. of treatment
Urokinase	310	40
Heparin	545	20
EDTA	0	0
Trypsin	150	5

EXAMPLE 4 - M-EDTA AND THE PREVENTION OF BIOFILM FORMATION

The present example is provided to demonstrate the utility of the M-EDTA preparation in preventing glycocalyx-rich biofilm formation at the surface of a medical device, as well as to demonstrate the anti-staphylococcal activity of minocycline and EDTA preparations.

The method of Example 3 was used with the following modifications:

1. A more intense exposure to staphylococci (*S. epidermidis* and *S. aureus*) was achieved by flushing the MRD for 6 hours (instead of 3 hours in Example 3) with $3x10^6$ CFU of staphylococci/ml of D_5/W; and

2. The growth of adherent staphylococci to the catheter segments was promoted and achieved by exposing the catheter segments at 37°C to a 10% broth solution (prepared by adding 1 ml of trypticase soy broth to 9 ml of sterile H_2O) of EDTA (30 mg/ml of 10% broth solution), heparin (100 units/ml of 10% broth), urokinase (5,000 units/ml of 10% broth), minocycline (3 mg/ml of 10% broth), mino/EDTA (3 mg/30 mg per ml of 10% broth), vancomycin (3 mg/ml of 10% broth), vancomycin/heparin (3 mg vancomycin plus 100 units heparin/ml of 10% broth), or D_5/10% broth (50 mg/ml of 10% broth solution).

The results from these studies are demonstrated at Table 3 (*S. epidermidis*) and Table 4 (*S. aureus*).

TABLE 3

NO. OF ADHERENT *S. EPIDERMIDIS* COLONIES OBTAINED FROM 0.3 CM^2 CATHETER SURFACES

Agent Used	After 4 hrs. of treatment	After 24 hrs. of treatment
Urokinase	$>5x10^3$	$>5x10^3$
Heparin	$>5x10^3$	$>5x10^3$
EDTA	800	20
Minocycline	10	0
Minocycline/EDTA	0	0
Vancomycin	55	85
Vancomycin/Heparin	445	40
D_5/10% broth	$>5x10^3$	$>5x10^3$

As demonstrated in Table 3, the urokinase, heparin and dextrose solutions alone were equally ineffective in preventing and eradicating *S. epidermidis* adherence after 4 or 24 hours of catheter treatment. The minocycline and minocycline/EDTA provided effective prevention and

irradication of *S. epidermidis* adhesion after only 4 hours of treatment. Minocycline/EDTA was slightly more effective than minocycline alone at 4 hours. EDTA alone and vancomycin/heparin provided minimal prevention at 4 hours but were more effective after 24 hours. Vancomycin alone provided equal partial prevention at 4 and 24 hours. M-EDTA was superior to all preparations examined, including vancomycin, vancomycin/heparin, minocycline or EDTA alone.

TABLE 4

NO. OF ADHERENT *S. AUREUS* COLONIES OBTAINED FROM 0.3 CM^2 CATHETER SURFACES

Agent Used	After 4 hrs. of treatment	After 24 hrs. of treatment
Urokinase	$>5x10^3$	$>5x10^3$
Heparin	256	$>5x10^3$
EDTA	750	30
Minocycline	0	0
Minocycline/EDTA	0	0
Vancomycin	605	230
Vancomycin/Heparin	140	185
D_5/10% broth	$>5x10^3$	$>5x10^3$

* All staphylococcus isolates were bloodstream slime-producing isolates obtained from human patient cases with catheter-related bacteremia.

Table 4 demonstrates that minocycline and minocycline/EDTA solutions were the most effective inhibitors of *S. aureus* adhesion, with 0 adherent colonies being observed after 4 hours of treatment. EDTA alone, vancomycin alone and vancomycin/heparin were significantly less effective for preventing adherent *S.*

aureus. These later three preparations had some partial anti-adherent activity, particularly after 24 hours of treatment.

These data (Table 3 and Table 4) demonstrate that minocycline alone or in combination with EDTA was effective for inhibiting *S. epidermidis* and *S. aureus* adherence and colonization of a catheter surface.

EXAMPLE 5 - M-EDTA AND THE INHIBITION OF *C. albicans* ADHESION

The present example is provided to demonstrate the utility of the M-EDTA formulation in the inhibition of other glycocalyx and biofilm-forming microorganisms, such as *C. albicans*.

The M-EDTA flush formulation described in Example 1 was employed in the present example. The method employed was the same as that described at Example 4, with the following modifications. The organism used was a *C. albicans* obtained from the bloodstream of a patient with catheter-related candidemia. The infected infusate consisted of D_5/W with $4x10^2$ CFU of *C. albicans* per ml flushed through the MRD for 6 hours. Results from the study are presented in Table 5.

TABLE 5

NO. OF ADHERENT *C. ALBICANS*

OBTAINED FROM 0.3 CM^2 CATHETER SURFACES

Agent Used	After 4 hrs. of treatment	After 24 hrs. of treatment
Urokinase	$>5x10^3$	$>5x10^3$
Heparin	$>5x10^3$	$>5x10^3$
EDTA	1060	155
Minocycline	190	535
Minocycline/EDTA	0	0
D_5/10% broth	$>5x10^3$	$>5x10^3$
Vancomycin/heparin	$>5x10^3$	$>5x10^3$
Vancomycin	470	$>5x10^3$

This example demonstrates minocycline/EDTA as a unique antistaphylococcal and antifungal agent. Vancomycin (a standard antistaphylococcal agent) when used alone or with heparin failed to have any anti-*C. albicans* activity and was not different from dextrose, urokinase or heparin solutions against *C. albicans*.

EDTA alone had some anti-*C. albicans* activity after 24 hours and minocycline alone had some activity at 4 and probably 24 hours. The combination of minocycline and EDTA (M-EDTA) provided a synergistic enhancement of anti-*C. albicans* activity, demonstrating an essentially total inhibitory effect against fungal adherence after 4 and 24 hours exposure. Therefore, M-EDTA is unique in preventing staphylococcal and Candida adherence to catheter surfaces (Staphylococci and Candida contributing to 95% to 100% of the pathogenic microbiology of catheter-related infections).

These results demonstrate that the solutions of a mixture of minocycline and EDTA provide a more effective

and rapidly-acting preparation for the prevention of *S. epidermidis*, *S. aureus*, and *C. albicans* adhesion to a catheter surface than any other thrombolytic (urokinase), anticoagulant (heparin, EDTA), or antistaphylococcal preparation (minocycline, vancomycin, vancomycin/heparin).

EXAMPLE 6 - *S. epidermidis* BIOFILM FORMATION AND HEPARIN, UROKINASE AND DEXTRINASE TREATMENT

The present example is provided to examine the relative *S. epidermidis* glycocalyx biofilm-destroying activity of heparin, urokinase and dextranase as assessed by scanning electron microscopy of an *S. epidermidis*-colonized catheter surface.

Scanning electron microscopy was done on various segments of a catheter exposed to *S. epidermidis* and then later exposed to heparin, urokinase or dextranase for 24 hours. A reduction in biofilm (glycocalyx) was noted on colonized catheter surfaces exposed to EDTA for 24 hours, compared to colonized surfaces later exposed to heparin, urokinase, or dextranase for 24 hours (Figure 1 = Dextranase; Figure 2 = Urokinase; Figure 3 = EDTA; Figure 4 = Saline).

EXAMPLE 7 - PRETREATMENT OF CATHETER SURFACES WITH EDTA, Dextranase OR SALINE AND *S. epidermidis* BIOFILM FORMATION

The present example is provided to demonstrate the effect of chemically pretreating a catheter surface with EDTA or dextranase, compared to a saline control, on the formation of polysaccharide-rich biofilm formation, such as that characteristic of *S. epidermidis*, and the adhesion of these organisms to a catheter surface.

Catheter surfaces were coated with EDTA, dextranase or control (saline), at the concentrations described in Example 5, and then exposed to slime producing *S. epidermidis*.

No biofilm or organisms were observed on EDTA coated surfaces. However, biofilm formation was observed on catheter surfaces pretreated with dextranase (Figure 4 and 5 = saline; Figure 3 = EDTA; Figure 6 = Dextranase pretreated).

EXAMPLE 8 - MINOCYCLINE COATING OF A CATHETER AND MICROBIAL COLONIZATION

The present example is provided to demonstrate the anti-microbial colonization effect of the use of minocycline at a catheter surface.

Catheter surfaces were coated with minocycline, vancomycin or control cement with H_2O. Catheter surfaces were then exposed to clinical staphylococci isolates. The Modified Robbin's Device was employed in this study (See Example 2. The Modified Robbin's Device simulates a vascular catheter, and therefore provides a model predictive of *in vivo* effects.

One gram of methylmethacrylate (cement) was mixed with 0.5 ml of sterile H_2O and one of the following:

1. 60 mg of minocycline
2. 60 mg of vancomycin
3. control (cement + H_2O alone)

Equal amounts of cement alone or with minocycline or vancomycin were put in the lumen of catheter latex segments in a specimen plug of the Modified Robbin's

Device. Twenty-four hours later, a one-liter infusate bag made of 5% dextrose in water was infected with 5 ml of 10^5 to 10^8 colony forming units (CFU) per ml of slime producing *Staphylococcus epidermidis* strains obtained from the bloodstream of patients with catheter related bacteremia. Using a peristaltic pump, the infected infusate was run for 2 hours at a rate of 60 ml/hr through the catheter segments of the Modified Robbin's Device.

Each catheter segment was made of 30 mm^2 silicone with a lumen filled with cement. At the end of 2 hours, some catheter segments (control and antibiotics coated) were taken out from specimen plugs and the cement in the lumen was removed, then the surface that was exposed to the infected fluid was cultured semiquantitatively using the roll-plate technique. Other segments were left behind and flushed with saline solution for 4 hours, then cultured by roll-plate.

Electron microscopy was used to document the adherence of staphylococci and the formation of biofilm layer on the surface of control uncoated catheter segments. Leaching of antibiotics from the cement was demonstrated to occur for at least one week by determining the inhibition around disc-shaped pieces of cement placed on blood agar plates that had been inoculated with bacteria. Coating of the catheter segments with antibiotics was demonstrated by the zone of inhibition that continued to form for at least one week around the disc-shaped catheter segments (without cement) placed on agar plates that had been inoculated with bacteria. The results from this study are presented in Table 6.

TABLE 6

NO. COLONIES OF S. EPIDERMIDIS

FROM 30 MM^2 CATHETER SURFACE

Coating	Before Flush	After Flush
Control	336	128
Vancomycin	174	111
Minocycline	48	15

Catheter segments coated with minocycline had a significantly lower number of adherent *Staphylococcus epidermidis* colonies, compared to control and vancomycin coated catheter segments (see Table 6). However, fibrous glycocalyx was not inhibited on the minocycline-coated catheter surfaces. The scanning electron microscopic figures also evidenced these findings. See Figure 7 - shows fibrous glycocalyx on the surface of a control catheter segment - before flush; Figure 8 - shows some deranged fibrous glycocalyx on the surface of minocycline coated catheter segment - before flush; Figure 9 - shows fibrous glycocalyx on the surface of another control (saline) catheter segment after flush; and Figure 10 - shows fibrous glycocalyx on the surface of minocycline coated catheter segment after flush.

These data demonstrate that the coating of catheters with minocycline alone significantly reduced staphylococcal adherence. Fibrous glycocalyx formation is not inhibited with minocycline-coated surfaces.

EXAMPLE 9 - STABILITY OF MINOCYCLINE AND EDTA PHARMACEUTICAL PREPARATIONS

The present studies will be conducted to characterize the stability of the pharmaceutical M-EDTA flushing solutions of the present invention. The M-EDTA

solutions are expected to retain their potency for relatively long periods of time when stored refrigerated at about 4°C, i.e., for at least 1 month.

The solutions of the present invention have been examined for retained potency at room temperature (37°C). The M-EDTA solutions have been observed to retain relatively full anti-microbial and anti-fungal potency for at least 72 hours at room temperature. It is therefore expected that the formulation has a shelf life that renders it suitable for routine hospital use (Table 1 and Figure 11).

EXAMPLE 10

COMPARATIVE CLINICAL TRIAL OF M-EDTA AND HEPARIN FOR THE PREVENTION OF CATHETER-RELATED INFECTIONS

The present example outlines a study wherein the relative effectiveness of an M-EDTA catheter flushing solution will be compared to a heparin flushing solution (a currently used standard preparation) for the prevention of catheter-related infections and occlusions in humans.

The objective of these studies is to further document the utility of the M-EDTA flush solution as compared to a heparin flush solution in preventing infection and/or occlusion in central venous catheters (CVC).

Eligibility for enrollment into these studies will be based on the following inclusion/exclusion criteria:

Inclusion Criteria

1. Patients must have a new (≤7 days old) functioning central venous catheter, utilized for infusion of chemotherapy, blood products, or other intermittent infusions.

2. Patients must be able to return to the outpatient clinic for evaluation in case of CVC occlusion or occurrence of fever.

3. Patients must have life expectancy for the planned duration of the study and must have catheter in place for study duration (study duration for a single patient is 6 months).

4. Catheters in the percutaneous/tunneled group will be limited to Hickman/Broviac.

Exclusion Criteria

1. Patients with an occluded central venous catheter.

2. Patients with any existing local or systemic catheter infection.

3. Patients with triple lumen catheters.

4. Patients with polyurethane or teflon catheters.

5. Patients currently taking warfarin.

6. Patients requiring previous catheter removal due to venous thrombosis.

7. It must be expected that the dwell time of 4 hours will not interfere with routine treatment of the underlying disease.

8. Patients with Groshong catheters.

Treatment Plan: Patients will be randomly assigned and in double blind manner to have their CVC flushed with either M-EDTA or heparin according to the following:

1. Tunneled CVC (Hickman/Broviac) will receive either
   (a) two mls of M-EDTA (containing 3 mg of minocycline and 30 mg EDTA/ml) q daily
   (b) two mls of Heparin (100U/ml) q daily.

2. Infusion ports will receive either
   (a) two mls of M-EDTA (3 mg minocycline and 30 mg EDTA/ml) q 3 weeks
   (b) two mls of Heparin (100U/ml) q 3 weeks

Endpoints and Treatment Evaluation: All patients will be followed up for 6 months and will be evaluated for 2 endpoints: catheter infection/colonization and occlusion. Catheter infection will include local CVC-related infection or systemic catheter-related septicemia. Catheter colonization will include positive quantitative catheter culture (flush technique) or positive quantitative blood culture through the CVC in the absence of a positive peripheral blood culture or clinical manifestations of sepsis (fever, chills or hypotension). Patients in the study who develop fever will be evaluated, and simultaneous quantitative blood cultures through CVC and peripheral vein conducted. Catheter occlusion will be categorized as complete or partial depending on whether one cannot withdraw blood,

infuse fluids through the CVC, or both. This subgroup of infected catheters will be analyzed separately.

Statistical Considerations: Based on a recent surveillance study conducted by the inventors (see Table 7), the rate of CVC-related sepsis in pediatric oncology patients ranges from 15% - 20.5% (see Table 6). These same ranges will be employed in further establishing the clinical utility of the treatment in adult patients.

TABLE 7

INFECTION RATES ASSOCIATED WITH HICKMAN/BROVIAC CATHETERS AND IMPLANTABLE PORTS IN PEDIATRIC ONCOLOGY PATIENTS, PER 100 CATHETERS

Type of Infection	Hickman/Broviac (N)	Port (N)	Total (N)
LOCAL CATHETER INFECTION			
Exit site or port infection	17.9 (7)	3.0 (3)	7.2 (10)
Extraluminal infection (tip ≥15 cfu)	0 (0)	0 (0)	0 (0)
Infection secondary to intraluminal colonization	7.7 (3)	3.0 (3)	4.3 (6)
Tunnel tract infection	2.6 (1)	N/A	0.7 (1)
Total	28.2 (11)	6.0 (6)	12.2 (17)
CATHETER-RELATED SEPSIS			
Definite	7.7 (3)	2.0 (2)	3.6 (5)
Probable & physician diagnosed	12.8 (5)	13.0 (13)	12.9 (18)
Total	20.5 (8)	15.0 (15)	16.5 (23)
# Catheters	N = 39	N = 100	N = 139

The results from this study will be employed in the development of a clinical protocol for the treatment and infection-free maintenance of indwelling catheters in humans.

EXAMPLE 11 - PREPARATION OF M-EDTA-COATED DEVICES FOR *IN VIVO* USE

The present example is provided to demonstrate the utility of the M-EDTA solution as a coating material for medical devices, most particularly catheters.

Any of a variety of coating techniques may be used for imparting a protective covering of the M-EDTA solution to a device. By way of example, such methods include emersion of a medical device, such as a catheter, into a solution of minocycline and EDTA. This example provides a description of how these particular M-EDTA coated catheters were prepared.

Bioguard Cook Catheters with TDMACC surfactant were immersed in antibiotic solutions containing the following:

1. 60 mg of Minocycline plus 60 mg of EDTA/ml
2. 60 mg of Minocycline/ml
3. 60 mg of EDTA/ml

Catheters were immersed in each of the three solutions listed above for about 15 minutes. Bioguard Cook catheters not treated with any of the 3 solutions listed above (which are not coated by antimicrobials) were used as negative controls. Arrow Gard catheters coated with chlorhexidine and silver sulfadiazine were used as positive control devices. The Arrow Gard catheter is coated with antimicrobials. This catheter is

described by Maki *et al.* in a clinical study, and reportedly decreased the rate of catheter-related bloodstream infection by five-fold as compared to a standard polyurethane triple-lumen CVC without a chlorhexidine and silver sulfadiazine coating.

Other Coating Methods for Medical Devices

As noted, preparations of the present invention may be advantageously used as a coating preparation for treating the surfaces of a medical device. The medical devices which are amendable to coatings with the subject M-EDTA preparations generally have surfaces composed of thermoplastic or polymeric materials such as polyethylene, Dacron, nylon, polyesters, polytetrafluoroethylene, polyurethane, latex, silicone elastomers and the like. Devices with metallic surfaces are also amenable to coatings with the disclosed combinations. Such devices, for example indwelling catheters of types listed herein, can be coated by cement mixture containing the subject antibiotic compounds. Alternatively, devices that include a surfactant, such as the Bioguard Cook catheters, may be used whereby the surfactant at the surface of the device allows for the coating of the material onto the device. Particular devices especially suited for application of the M-EDTA preparation include intravascular, peritoneal, pleural and urological catheters; heart valves; cardiac pacemakers; vascular shunts; and orthopedic, intraocular, or penile prosthesis.

Various methods can be employed to coat the surfaces of medical devices with the M-EDTA coating preparation described herein (60 mg EDTA/60 mg minocycline). For example, one of the simplest methods would be to flush the surfaces of the device with the M-EDTA preparation.

Generally, coating the surfaces by a simple flushing technique would require convenient access to the implantable device. For example, catheters, are generally amenable to flushing with a solution of EDTA and minocycline. For use as coating solutions, the effective concentration of minocycline would range from about 0.001 to 100 mg/ml, preferably about 60 mg/ml; and about 1 to 100 mg/ml EDTA, preferably about 60 mg/ml EDTA. The coating solution would normally be further composed of a sterile water or a sterile normal saline solutions.

A preferred method of coating the devices would be to first apply or adsorb to the surface of the medical device a layer of tridodecylmethyl ammonium chloride (TDMAC) surfactant followed by a coating layer of the M-EDTA preparation. This method for coating a device with M-EDTA provides for first absorbing to the catheter surface a cationic surfactant, such as TDMAC, to medical devices having a polymeric surface, such as polyethylene, silastic elastomers, polytetrafluoroethylene or Darcon, by soaking in a 5% by weight solution of TDMAC for 30 minutes at room temperature. The device should then be air dried, and rinsed in water to remove excess TDMAC. Alternatively, TDMAC pre-coated catheters are commercially available; for example, arterial catheters coated with TDMAC are available from Cook Critical Care, Bloomington, Indiana. The device carrying the adsorbed TDMAC surfactant coating can then be incubated in a solution of the M-EDTA combination for between about 15 minutes and an hour, washed in sterile water to remove unbound M-EDTA and stored in a sterile package until implantation. In general, the soaking solution of M-EDTA includes between about 10 mg/ml - 100 mg/ml EDTA (preferably about 60 mg/ml) and between about 10 mg/ml -

100 mg/ml minocyclines (preferably about 60 mg/ml) in an aqueous pH 7.4-7.6 buffered solution or sterile water.

Alternative processes and reagents for bonding an agent contained in a solution to a surfactant-coated implantable medical device are provided in U.S. Patent Nos. 4,442,133, 4,678,660 and 4,749,585, which patents are incorporated herein in their entirety by reference for this purpose. A further method useful to coat the surface of a medical device with an M-EDTA preparation involves first coating the selected surfaces with benzalkonium chloride followed by ionic bonding of the M-EDTA. *See, e.g.*, Solomon, D.D. and Sherertz, R.J. (1987)[17] and U.S. Patent No. 4,442,133[18].

Other methods of coating surfaces of medical devices with antibiotics are taught in U.S. Patent No. 4,895,566 (a medical device substrate carrying a negatively charged group having a pKa of less than 6 and a cationic antibiotic bound to the negatively charged group); U.S. Patent No. 4,917,686 (antibiotics are dissolved in a swelling agent which is adsorbed into the matrix of the surface material of the medical device); U.S. Patent No. 4,107,121 (constructing the medical device with ionogenic hydrogels, which thereafter absorb or ionically bind antibiotics); U.S. Patent No. 5,013,306 (laminating an antibiotic to a polymeric surface layer of a medical device); and U.S. Patent No. 4,952,419 (applying a film of silicone oil to the surface of an implant and then contacting the silicone film bearing surface with antibiotic powders).

These and many other methods of coating a solution to a solid surface appear in numerous patents and medical journal articles. As is evident, one of ordinary skill having benefit of this disclosure would be apprised of

several different methods of coating a medical device surface with the subject inventive minocycline and EDTA coatings.

Medical devices, particularly catheters of the type listed in Table 8, may be coated with the M-EDTA solution and then stored in a sterile packaging material until use.

TABLE 8

SHORT-TERM TEMPORARY ACCESS CATHETER	LONG-TERM INDEFINITE VASCULAR ACCESS
Peripheral intravenous cannulas - winged steel needles - peripheral intravenous catheters	Peripherally inserted central venous catheters (PICC)
Arterial catheters	Percutaneous nontunneled silicone catheters
Central venous catheters	Cuffed tunneled central venous catheters (Hickman and Broviac)
Swan-Ganz catheters	Subcutaneous central venous ports (Infusaport, Port-a-cath, Landmark)
Hemodialysis catheters	
Umbilical catheters	

EXAMPLE 12

METHOD FOR MAINTAINING CATHETER PATENCY WITH MINOCYCLINE-EDTA PHARMACEUTICAL PREPARATION

The present example demonstrates one embodiment of a method that may be used in maintaining the patency of an indwelling catheter in a patient. The regimen described herein is potentially applicable for use in adult

patients, as the dose of M-EDTA in the regimen exposes patients only to relatively low, pharmaceutically acceptable levels of the EDTA and minocycline, and has already been demonstrated by the inventors as effective in pediatric patients.

An indwelling catheter of a patient will be flushed with a solution of minocycline/EDTA. The "flushing" of the catheter will constitute filling the catheter with a volume of the M-EDTA solution sufficient to provide a concentration of about 9.0 mg minocycline and a concentration of about between 90 mg EDTA in the catheter. For example, assuming a catheter volume of about 2-3 ml., the solution will contain a concentration of EDTA of between about 10 mg/ml - 30 mg/ml, and a concentration of minocycline of between about 1-3 mg/ml. "Flushing" the catheter with about 3 ml of the M-EDTA solution will thereby provide a dose of between 3-9 mg minocycline and about 30 - 90 mg EDTA. The solution of M-EDTA will be prepared as outlined in Example 1.

The "flushing" of the catheter is achieved by adding between 2-3 ml of the M-EDTA solution to the catheter. The solution is then allowed to diffuse through the catheter to the patient in which it is implanted. The concentration of the EDTA and minocycline in the solution is such that the patient will be exposed only to concentrations of the agents well below pharmacologically tolerable levels.

The flushing of the catheter is to be repeated at periodic intervals of at least between every 24 to 72 hours (preferably, every 24 hours) to assure that infectious organisms are not allowed an opportunity to colonize the surface or initiate biofilm formation on the catheter surface.

EXAMPLE 13

EFFICACY OF ANTIBIOTIC COATED CATHETERS AFTER GAS STERILIZATION

The present example is provided to demonstrate the stability of the coated devices to sterilization processes. In order to test the effect of gas sterilization on catheters coated with Minocycline, EDTA and the combination of drugs, the following studies were performed.

M-EDTA-coated catheters were prepared as described in Example 11. The catheters were then divided into three representative groups. The catheter antibiotic activity was determined in vitro by a modified Kirby-Bauer technique described in Sherertz *et al.* ((1989) *Antimicrob. Agents Chemother.*, 33:1174-1178), which reference is specifically incorporated herein by reference for this purpose. The first set of catheters were tested immediately after immersion without gas sterilization. The second set was tested 24 hours later without gas sterilization. The third set was tested 24 hours after gas sterilization.

The modified Kirby-Bauer technique consisted of growing a strain of slime producing catheter-related bacteremic isolate of *Staphylococcus epidermidis* for 18 hours in trypticase soy broth, then diluting the solution to 10 CFU ml in phosphate-buffered saline. A cotton swab was placed in the staphylococcal suspension and then rubbed across the surface of a trypticase soy agar plate. Individual catheters were cut into 20 mm lengths pressed into agar overlaid with *S. epidermidis* and incubated overnight at 37°C. Zone sizes were assessed by measuring the diameter perpendicular to the long axis of the catheter. The data in Table 9 demonstrates that

Catheters treated with the M-EDTA preparations maintained the greatest post-sterilization zone of inhibition (0 hours =40; 24 hours = 34).

For the *in vivo* studies, the catheters were cut into 2 cm segments and then emersed in 60 mg/ml EDTA, 60 mg/ml minocycline solution each and then immersed in a solution of a mixture containing 60 mg/ml mino and 60 mg/ml EDTA for 15 minutes. All catheters were allowed to dry for one hour and they were gas sterilized.

Table 9 provides the results achieved with these studies. This data demonstrates the superior anti-*S. epidermis* activity of the M-EDTA coating as compared to non-treated as well as the Arrow Gard catheters.

TABLE 9 *S. EPIDERMIDIS*

ZONE OF INHIBITION - DIAMETER (mm)

	Pre Sterilization		Post Sterilization
	0 Hrs	24 Hrs	24 Hrs
Minocycline	36	33	18
M-EDTA	40	34	35
EDTA	5	18	10
Control	0	0	0
Arrow Gard*	13	15	7

*Coated with chlorhexidine gluconate and silver sulfadiazine

EXAMPLE 14

EFFICACY OF ANTIBIOTIC COATED CATHETERS AFTER GAS STERILIZATION TO *S. AUREUS*

The present example is provided to further demonstrate the stability of the described M-EDTA coatings to sterilization processes, such as gas sterilization, particularly as measured through the retained anti-microbial activity of the device.

The protocol of Example 13 was used to prepare the catheters used in this study (using a catheter-related bacteremic strain of *S. aureus*). The 0 hour catheters, and catheters coated with minocycline or EDTA alone were not included in the study.

TABLE 10 *S. AUREUS*

ZONE OF INHIBITION - DIAMETER (mm)

	Pre Sterilization	Post Sterilization
	24 Hrs	24 Hrs
M-EDTA	31	29
Control	0	0
Arrow Gard*	13	13

*Coated with chlorhexidine gluconate and silver sulfadiazine

The present example demonstrates that gas sterilization did not affect the antibiotic activity of minocycline-EDTA coated catheters and that these catheters were at least two times more active at inhibiting *Staphylococcus aureus* than the Arrow septic catheters.

EXAMPLE 15

COMPARATIVE EFFICACY OF M-EDTA COATED CATHETERS AGAINST CATHETER RELATED MICROORGANISMS

The present example demonstrates the broad spectrum activity of M-EDTA catheters. The same zone of inhibition measure described in examples 13 and 14 (with the modified Kirby-Bauer technique) were employed using M-EDTA coated catheters and Arrow Gard catheters (commercially available) to examine the effectiveness of the various coatings against different catheter-related organisms such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Candida albicans* and gram negative bacilli (*Pseudomonas aeruginosa*, *Xanthomonas maltophilia*, and acinetobacter species.) Approximately 60% of catheter infections are caused by *S. epidermidis*, 10% by *S. aureus*, 10% by *C. albicans*, 20% by gram negative bacilli (mostly *P. aeruginosa*, *X. maltophilia*, and acinetobacter species.) These studies show that catheters coated with M-EDTA have a broad spectrum activity against different species of bacteria and fungi as well as different strains of the same species. For purposes of the present studies, a zone of inhibition of ≥ 15 mm is a predictor of excellent efficacy in veins. A zone of 10 - 15 mm is a predictor of moderate efficacy and a zone of inhibition of ≤ 10 mm is a predictor of poor efficacy.

Staphylococcus epidermis

The data in Table 11 demonstrates that the M-EDTA coated catheters has a significantly greater zone of inhibition to five strains of staphylococcus epidermidis, as compared to non-M-EDTA coated catheters (Arrow Gard catheters).

TABLE 11 - ZONES OF INHIBITION (mm)
(STAPHYLOCOCCUS EPIDERMIDIS (SE) STRAINS

Strain No.	Arrow Gard	Mino/EDTA
SE 4392	14	31
SE 3996	16	29
SE 4345	17	39
SE 4023	15	29
SE 93	15	31
Mean (SD)	15.4 (±1.1)	31.8 (±4.14)

p = 0.001

Staphylococcus aureus

Table 12 sets forth data obtained employing five different strains of *Staphylococcus aureus* (SA) in the aforedescribed zone of inhibition assays. The data demonstrate that the M-EDTA coated catheters provided a significantly greater zone of inhibition compared to the non-M-EDTA catheters (Arrow Gard).

TABLE 12 - ZONES OF INHIBITION - DIAMETER (mm)

Strain No.	Arrow Gard	Mino/EDTA
SA 1445	13	23
SA 1432	15	28
SA 1414	12	23
SA 48	14	23
SA 1411	12	34
Mean (SD)	13.2 (±1.3)	26.2 (±4.9)

p = 0.005

Candida albicans

Table 13 sets forth data obtained employing the organism *Candida albicans*, in the zone of inhibition

study protocol, comparing the inhibitory action of the M-EDTA and non-M-EDTA coated catheters especially. "Ampho B" stands for a broad spectrum antibiotic **(INVENTORS, PLEASE SUPPLY)**. The data in Table 13 demonstrates that the M-EDTA treated catheters had superior anti-*Candida albicans* inhibitory activity as compared to control and the three other catheter types (coatings) tested.

TABLE 13 - ZONES OF INHIBITION - DIAMETER (mm)

Candida albicans

	Trial #1	Trial #2	Trial #3
M-EDTA	16	21	16
Minocycline	0	0	0
Control	0	0	9
Arrow Gard	10	9	9
Ampho B	ND	18	18

Table 14 sets forth data obtained in studies with five strains of *Candida albicans*, and again demonstrates the broad range anti-microbial activity of the M-EDTA coated catheters.

TABLE 14 - ZONES OF INHIBITION - DIAMETER (mm)
(STRAINS OF *CANDIDA ALBICANS* (CA)

Strain No.	Arrow Gard	Mino/EDTA
CA 291	0	16
CA 596	10	12
CA 276	10	10
CA 267	10	13
CA 319	7	18
Mean (SD)	7.4 (±4.3)	13.8(±3.2)

p = 0.030

Acinetobacter (Acin)

Table 15 demonstrates the efficacy of the described M-EDTA coatings for inhibiting Acinetobacter.

TABLE 15 - ZONES OF INHIBITION - DIAMETER (mm)

Strain No.	M/EDTA	TMP/SMX	CFTZ	ARROW GARD
ACIN639	30	00	00	00
ACIN38B	23	30	15	05
ACIN632	24	10	00	00
ACIN633	10	15	00	09
ACIN1771	43	14	00	12
Mean*	26	14	3.0	5.2
STDEV	12	11	6.7	5.3

M = Minocycline; CFTZ = Ceftazidime;
TMP-SMX = Trimethoprin-Sulfamethoxazole

* The efficacy of the catheter coated with M-EDTA was significantly higher than the Arrow Gard (p = 0.016)

P. Aeruginosa (PSA) Strains

Table 16 demonstrates the efficacy of the M-EDTA coated catheters against *P. aeruginosa* (PSA) strains.

TABLE 16 - ZONES OF INHIBITION - DIAMETER (mm)

Strain No.	M/EDTA	TMP/SMX	CFTZ	ARROW GARD
PSA1644	15	00	13	03
PSA2455	11	05	20	06
PSA2451	11	00	20	06
PSA2456	14	05	29	00
PSA2452	10	00	28	06
Mean*	12.2	1.0	22	3.0
STDEV	2.17	2.24	6.60	3.0

* The efficacy of the catheter coated with M-EDTA was significantly higher than the Arrow Gard ($p = 0.009$)

X. Maltophilia (XMAL) Strains

Table 17 demonstrates the efficacy of the M-EDTA catheters against *X. maltophilia* strains.

TABLE 17 - ZONES OF INHIBITION - DIAMETER (mm)

Strain No.	M/EDTA	TMP/SMX	CFTZ	ARROW GARD
XMAL5653	24	37	32	12
XMAL2496	34	22	29	00
XMAL8929	37	40	20	00
XMAL2657	35	30	15	04
XMAL2172	15	15	20	00
Mean*	29	28.8	23.3	3.0
STDEV	9.3	10.38	7.05	5.22

* The efficacy of the catheter coated with M-EDTA was significantly higher than the Arrow Gard ($p = 0.0016$)

The aforegoing results demonstrate that M-EDTA coated catheters have a broad spectrum activity against various microbial agents that can cause CVC related infections. This activity in the M-EDTA coated catheters is superior and provides a broader spectrum anti-microbial activity than that provided using Arrow Gard catheters.

EXAMPLE 16

SHELF LIFE AND STABILITY OF THE COATED M-EDTA (IN SERUM)

Catheters coated with M-EDTA, as well as control and Arrow catheters were tested at baseline (day 1 - D1) against *S. epidermidis* using a zone of inhibition measure for relative anti-microbial activity. Then the same

catheters were placed in serum at 37°C and tested at days 3, 7, 15 and 30 to determine inhibitory efficacy over time. (Table 17). In addition, segments of the same catheters were kept for 30 and 60 days at 25°C then tested to determine anti-microbial activity as a measure of the shelf life of cetheters with such coatings (Table 18).

STABILITY OF M-EDTA COATED CATHETERS

TABLE 17 - ZONES OF INHIBITION - DIAMETER (mm)

	D1	D3	D7	D15	D30
M-EDTA	31	21	16	14	10
Control	0	0	0	0	0
Arrow Gard	14	07	07	05	03

TABLE 18 - ZONES OF INHIBITION - DIAMETER (mm)

	DAY 30		DAY 60
	Serum 37°C	25°C	25°C
M-EDTA	10	34	32
Control	0	0	0
Arrow Gard	3	13	12

The foregoing studies demonstrate that M-EDTA coated catheters maintain excellent anti-microbial (particularly anti-S epidermidis) efficacy for at least two weeks in serum at 37°C and at least two months at 25°C. Whereas, the efficacy of the Arrow Gard catheters decreases rapidly within three days in serum (37°C).

EXAMPLE 17

IN VIVO EFFICACY OF M-EDTA COATED CATHETERS IN A RABBIT MODEL

Animal studies with polyurethane catheters coated with minocycline plus EDTA are provided. The results are consistent with the extensive *in vitro* data described herein. Currently, the only antibiotic-coated catheters on the market are those produced by Arrow. These catheters are coated with chlorhexidine gluconate and silver sulfadiazine. They have been described in a clinical study done by Maki *et al.* as capable of reducing the rate of catheter-related sepsis five fold.

An established *in vivo* rabbit model was used in the present example (Sherertz *et al. Journal of Infectious Diseases* 167:98-106, 1993). Catheters were inserted percutaneously in the rabbit under aseptic conditions. Immediately after insertion, the catheter insertion site was inoculated with 0.1 ml of 10^5 colony forming units (CFU) of *Staphylococcus aureus* from the bloodstream of a patient with catheter-related *S. aureus* bacteremia. Seven days after insertion, the catheters were removed and the subcutaneous (SQ) as well as the tip of the catheters were cultured by quantitative catheter cultures.

PREPARATION OF M-EDTA COATED CATHETERS

Catheters were first pretreated with a cationic surfactant tridodecylmeylammonium chloride (TDMAC). (Example 11). Other surfactants, such as benzalkonium chloride, may also be used. Treatment of a catheter with a surfactant will enable subsequent bonding of anionic substances, such as the antibiotic minocycline (mino) and EDTA to the surface. For the present studies,

polyethlylene catheters already coated with TDMAC were immersed in a 60 mg/ml solution of EDTA and 60 mg/ml minocycline, (Bio-Guard AB coating, Cook Critical Care, Bloomington, Ind.). These polyethylene catheters were manufactured by Cook Critical Care (Bloomington, Ind.), 5.0 Fr (18 ga), 15 cm (5 7/8 in)). For these *in vivo* studies, the catheters were cut into 6 cm segments before treating with the M-EDTA solution.

Solution A. 3 vials of minocycline (100 mg each) diluted with 0.8 ml of sterile water for injection, USP to obtain 2.4 ml of 120 mg/ml minocycline;

Solution B. 1.6 ml of 150 mg/ml EDTA was added to 0.4 ml sterile water to obtain 2 ml of 120 mg/ml EDTA. Finally, 2 ml of solution A was added to 2 ml solution B, resulting in a 4 ml solution of 60 mg/ml minocycline and 60 mg/ml EDTA.

All catheters used were of the polyurethane type made by Arrow or Cook. The following results were obtain in the experiment where catheters were cultured by either the standard semiquantitative roll-plate culture technique (Maki *et al.*, 1977, *N. Engl. J. Med.*, 296:1305-1309) or the sonication (Sherertz *et al.*, 1990, *J. Clin. Microbiol.*, 28:76-82) technique.

The data in Tables 19 and 20 demonstrated the consistently inhibitory activity of the M-EDTA coated catheters against S. aureus colonization.

TABLE 19 - NUMBER OF *S. AUREUS* COLONIES CULTURED BY ROLL-PLATE

Catheter Type	Catheter Tip	Catheter SQ*
Control	>1000	>1000
Control	>1000	>1000
Arrow Gard†	2	10
Arrow Gard	0	0
Arrow Gard	8	10
Arrow Gard	2	8
M-EDTA	0	0
M-EDTA	0	0

† Coated with chlorhexidine gluconate and silver sulfadiazine

* Subcutaneous catheter segment

TABLE 20 - NUMBER OF *S. AUREUS* COLONIES CULTURED BY ROLL-PLATE

Catheter Type	Catheter Tip	Catheter SQ*
Control	>1000	>1000
Control	18	25
Arrow Gard†	>1000	>1000
Arrow Gard	●	16
M-EDTA	0	0
M-EDTA	0	0

The data in Table 21 was obtained using catheter segments cultured by the quantitative sonication technique (as described by Sheretz *et al*. (1990). This data demonstrates again the conssitently anti-S. aureus colonizing effect provided through coating a catheter or other device with a combination of minocycline and EDTA.

TABLE 21 - NUMBER OF *S. AUREUS* COLONIES CULTURED BY SONICATION

Catheter Type	Catheter Tip	Catheter SQ*
Control	40	60
Control	380	>1000
Arrow Gard†	180	>1000
Arrow Gard	0	20
M-EDTA	0	0
M-EDTA	0	0

These studies demonstrate the complete prevention of colonization upon using M-EDTA coated catheters compared to the partial prevention achieved using the Arrow Gard catheters.

EXAMPLE 18

IN VIVO EFFICACY OF M-EDTA CATHETERS

The present example further demonstrates the anti-microbial activity of the coated M-EDTA devices *in vivo*. The M-EDTA-coated catheters were prepared as described in Example 17. 10^4 colony forming units of *S. aureus* were inoculated at the insertion site of a catheter coated with M-EDTA (as described in Example 13), with chlorhexidine gluconate and silver sulfadiazine (the Arrow Gard catheter) or with TDMAC alone with no added antibiotic.

The rabbit model described in Sherertz *et al.* (1993) was used in the present study, i.e., New Zealand white rabbits between 2-3 months old and weighing 2-3 kg. The data in Table 22 demonstrates the superior anti-staphylococcal and total inhibitory activity of MEDTA as

compared to the partial *anti-S. aureus* activity achieved with the Arrow Gard catheter.

TABLE 22- NUMBER OF *S. AUREUS* COLONIES CULTURED BY ROLL-PLATE

Catheter No.	Catheter Type	Catheter Tip	Catheter SQ	Catheter Site Purulence
1	Control	> 1000	> 1000	Yes
2	Control	> 1000	> 1000	Yes
3	Control	> 1000	> 1000	Yes
4	Control	> 1000	> 1000	Yes
5	Arrow Gard	3	10	No
6	Arrow Gard	15	10	No
7	Arrow Gard	15	15	No
8	Arrow Gard	20	15	No
9	M-EDTA	0	0	No
10	M-EDTA	0	0	No
11	M-EDTA	0	0	No
12	M-EDTA	0	0	No
13	M-EDTA	0	0	No
14	M-EDTA	0	0	No
15	M-EDTA	0	0	No
16	M-EDTA	0	0	No

EXAMPLE 19 - *IN VIVO* COMPARATIVE STUDY OF M-EDTA-COATED CATHETERS V. CHLORHEXIDINE/SILVER SULFADIAZINE-COATED CATHETERS

For this example, catheters were cultured by a sonication quantitative culture technique described in

Sheretz *et al*. (1990). The Sheretz *et al*. article is specifically incorporated herein by reference for this purpose. The sonication of technique is a quantitative culture technique, and is described in Raad *et al*. (1992) (Diagn. Microbiol. Infect. Dis., 15:13-20). The M-EDTA catheters were coated as described in Example 11. The chlorhexidine/silver sulfadiazine coated catheters are commercially available from Arrow International, Inc. (300 Bernville Road, Reading, PA 19605) (Arrow Gold catheters). The data in Table 23 again demonstrates the consistently effective anti-microbial, particularly the anti-S. aureus, activity of the M-EDTA coated devices of the invention. As well as the superior anti-microbial activity of the M-EDTA devices as compared to the Arrow Gard catheter.

TABLE 23 - NUMBER OF *S. AUREUS* COLONIES CULTURED BY SONICATION

Catheter Type	Catheter Tip	Catheter SO
Control	600	520
Control	400	480
Control	600	N/A
Control	640	N/A
Arrow Gard	40	80
Arrow Gard	120	80
Arrow Gard	0	160
Arrow Gard	80	160
M-EDTA	0	0
M-EDTA	0	0
M-EDTA	0	0
M-EDTA	0	0
M-EDTA	0	0
M-EDTA	0	0
M-EDTA	0	0
M-EDTA	0	0

EXAMPLE 20 - *IN VIVO* ANTI-MICROBIAL ACTIVITY OF M-EDTA COATED CATHETERS

This study was performed in the rabbit model as described in Example 18. 10^4 colony forming units (CFU) *of S. aureus* (a PI strain) were used to infect the catheter insertion site. The catheters were cultured by sonication. (Sheretz *et al.* (1990)). The Arrow Gard and Cook catheters coated with EDTA (Example 17) were employed in the study. The control catheters used were again the Cook catheters that have a coating of TIDMAC, but are without antibiotic or EDTA. Table 24 provides the date collected in this study. The results in Table 24 again demonstrate the *in vivo* effectiveness of the M-EDTA coated catheters for inhibiting infection by *S. aureus in vivo*.

Catheters were inserted into the subcutaneous space of New Zealand white rabbits 2-3 months old and weighing 2-3 kg. A 0.1 ml of 10 colonies of a virulent *S. aureus* strain (PI strain) was injected at the insertion site. The rabbits were sacrified on day 7. Catheters were aseptically removed and the 2 cm tip cultured by the sonication technique. The results are shown in Table 24. These results again demonstrate the *in vivo* effectiveness of M-EDTA against *S. aureus* infection. These results confirm previous experiments whereby M-EDTA coated catheters prevented staphylococcal colonization and catheter infection compared to partial prevention by the Arrow catheters.

TABLE 24 - NUMBER OF *S. AUREUS* COLONIES CULTURED BY SONICATION

Catheter No.	Catheter Type	Catheter Tip	Catheter Site Purulence
1	Control	$> 10^5$	Yes
2	Control	$> 10^5$	Yes
3	Control	$> 10^5$	Yes
4	Control	$> 10^5$	Yes
5	Arrow Gard	$> 10^3$	No
6	Arrow Gard	16	No
7	Arrow Gard	0	No
8	Arrow Gard	0	No
9	Arrow Gard	0	No
10	Arrow Gard	0	No
11	Arrow Gard	0	No
12	Arrow Gard	0	No
13	Arrow Gard	0	No
14	M-EDTA	0	No
15	M-EDTA	0	No
16	M-EDTA	0	No
17	M-EDTA	0	No
18	M-EDTA	0	No
19	M-EDTA	0	No
20	M-EDTA	0	No
21	M-EDTA	0	No
22	M-EDTA	0	No

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CLAIMS

1. An implantable medical device resistant to microbial growth and coated with at least one of EDTA or minocycline.

2. A catheter resistant to glycocalyx formation, said catheter being coated with at least one of EDTA or minocycline.

3. The implantable device of claim 1 or catheter of claim 2 coated with EDTA.

4. The medical device of claim 1 or catheter of claim 2 coated with minocycline.

5. The medical device of claim 1 or catheter of claim 2 coated with minocycline and EDTA.

6. The medical device of claim 1 or catheter of claim 2 defined further as prepared by a process of:

obtaining a medical device or catheter; and

treating the device or catheter surface with a preparation including at least one of minocycline or EDTA.

7. The medical device or catheter of claim 6 wherein the preparation includes minocyline and EDTA.

8. The medical device or catheter of claim 7 wherein the preparation includes between about 10 to about 100 mg/ml minocycline and between about 10 to about 100 mg/ml EDTA.

9. The medical device or catheter of claim 8 wherein the preparation includes about 60 mg/ml minocycline and about 60 mg/ml EDTA.

10. The medical device or catheter of claim 6 wherein the device or catheter is coated with a surfactant.

11. The medical device or catheter of claim 10 wherein the surfactant is tridodecylmethyl ammonium chloride.

12. The medical device or catheter of claim 10 wherein the surfactant is benzalkonium chloride.

13. The medical device of claim 1 or 6 or catheter of claim 2 or 6 further defined as a central venous catheter, a peripheral intravenous catheter, an arterial catheter, a Swan-Ganz catheter, a hemodialysis catheter, an umbilical catheter, a percutaneous nontunneled silicone catheter, a cuffed tunneled central venous catheter or a subcutaneous central venous port.

14. The medical device of claim 1 or 6 or catheter of claim 2 or 6 further defined as a urinary catheter or a peritoneal catheter.

15. A mixture of minocycline and at least about 10 mg/ml EDTA.

16. The mixture of claim 15 including between about 10 mg/ml and about 100 mg/ml EDTA.

17. The mixture of claim 15 including between about 0.001 mg/ml and 100 mg/ml minocycline.

18. The mixture of claim 17 including about 30 mg/ml EDTA and about 30 mg/ml minocycline.

19. The mixture of claim 17 including about 60 mg/ml EDTA and about 60 mg/ml minocycline.

20. The mixture of claim 15 including about 3 mg/ml minocycline and about 30 mg/ml EDTA in a saline carrier solution.

21. An EDTA solution for use in inhibiting polysaccharide glycocalyx formation comprising a solution containing at least about 10 mg/ml EDTA.

22. The EDTA solution of claim 21 including about 30 mg/ml EDTA.

23. A minocycline solution for use in preventing catheter infection containing between about 0.001 to about 100 mg/ml minocycline.

24. The minocycline solution of claim 23 including about 3 mg/ml minocycline.

25. An EDTA and minocycline solution for use in preventing catheter infection and polysaccharide gylcocalyx formation including between about 0.001 and 100 mg/ml minocycline and between about 10 and 100 mg/ml EDTA.

26. The EDTA and minocycline solution of claim 25 including about 30 mg/ml EDTA and about 3 mg/ml minocycline.

27. A method of preparing a glycocalyx-resistant medical device or catheter comprising coating the device or catheter with the mixture of claim 19 and allowing the coating to dry on the surface of the device or catheter.

28. The method of claim 27 wherein the device or catheter is an indwelling catheter.

29. The method of claim 27 wherein the device or catheter is resistant to formation of polysaccharide-rich glycocalyx.

30. The method of claim 29 wherein the device or catheter is resistant to formation of a staphylococcal glycocalyx.

31. The method of claim 27 wherein the device or catheter is a central venous catheter or a triple lumen catheter.

32. A method for preparing a biofilm-resistant medical device using a pharmaceutical preparation of minocycline and EDTA, comprising:

obtaining a medical device;

preparing preparation including at least about 10 mg/ml minocycline and at least about 10 mg/ml EDTA; and

treating the device surface with the pharmaceutical preparation to allow the formation of a film of the preparation on the device surface.

33. The method of claim 32 wherein the medical device is a catheter.

34. The method of claim 32 wherein the biofilm is a microbial glycocalyx.

35. The method of claim 32 wherein the biofilm is a polysaccharide-rich glycocalyx.

36. The method of claim 32 wherein the biofilm is a staphylococcal glycocalyx.

37. The method of claim 32 wherein the pharmaceutical preparation includes between about 10 to about 100 mg/ml minocycline and between about 10 to about 100 mg/ml EDTA.

38. The method of claim 32 wherein the pharmaceutical preparation includes about 60 mg/ml minocycline and about 60 mg/ml EDTA.

39. A method for inhibiting polysaccharide glycocalyx formation on a catheter comprising flushing the catheter periodically with a preparation including at least about 10 mg/ml EDTA in a pharmacologically acceptable carrier solution.

40. The method of claim 39 wherein the preparation includes about 10 mg/ml and about 100 mg/ml EDTA.

41. The method of claim 39 wherein the preparation includes minocycline.

42. The method of claim 39 wherein the preparation includes between about 0.001 mg/ml and about 100 mg/ml minocycline.

43. The method of claim 39 wherein the preparation includes about 30 mg/ml EDTA and about 3 mg/ml minocycline.

44. The method of claim 39 wherein the catheter is a tunneled catheter or an untunneled catheter.

45. The method of claim 39 wherein the catheter is to be flushed once every 4-24 hours.

46. A method for eliminating microbial glycocalyx formation at a catheter comprising:

preparing a solution of minocycline and EDTA in a carrier solution to provide an M-EDTA preparation; and

flushing the catheter periodically with the M-EDTA preparation.

47. The method of claim 46 wherein the solution includes about 0.001-100 mg/ml minocycline and about 10-100 mg/ml EDTA.

48. The method of claim 46 wherein the solution includes about 3 mg/ml minocycline and about 30 mg/ml EDTA.

49. The method of claim 46 wherein the microbial glycocalyx is a Staphylococcal glycocalyx.

50. The method of claim 45 wherein the Staphylococcal glycocalyx is an *S. epidermidis* glycocalyx or an *S. aureus* glycocalyx.

5

51. The method of claim 46 wherein the catheter is flushed once every about 24 hours with between about 1-10 ml of the solution of claim 48.

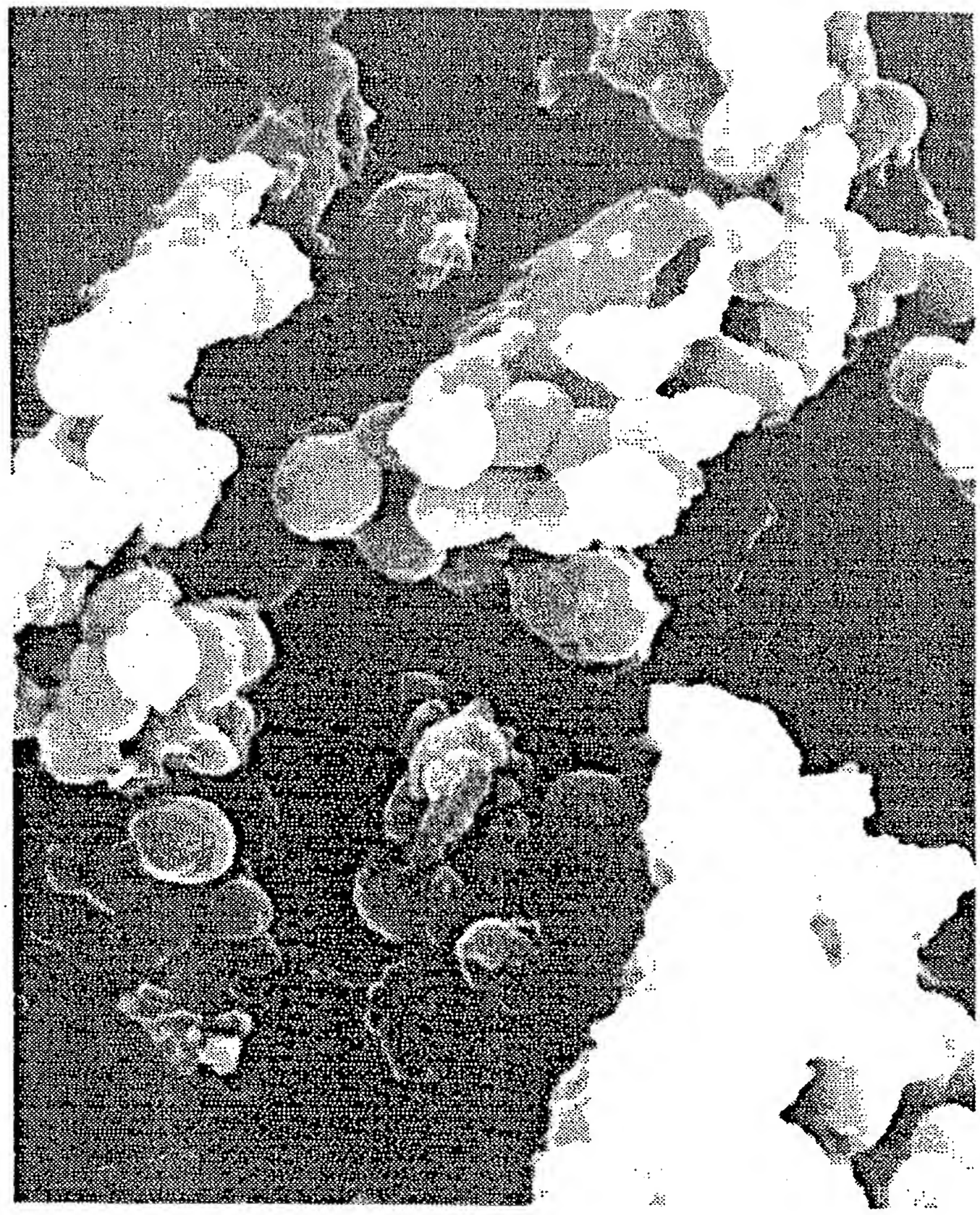

FIG. 1

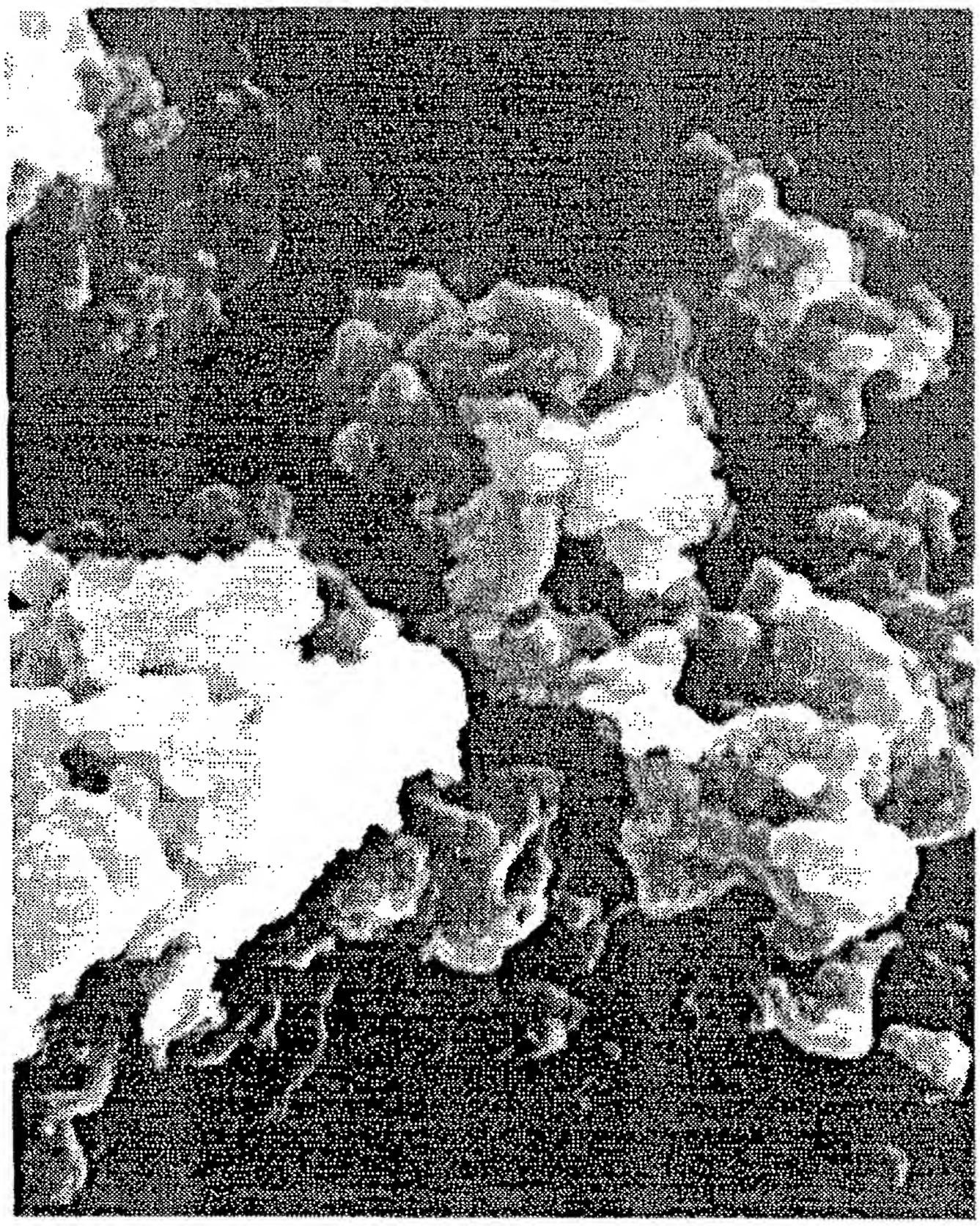

FIG. 2

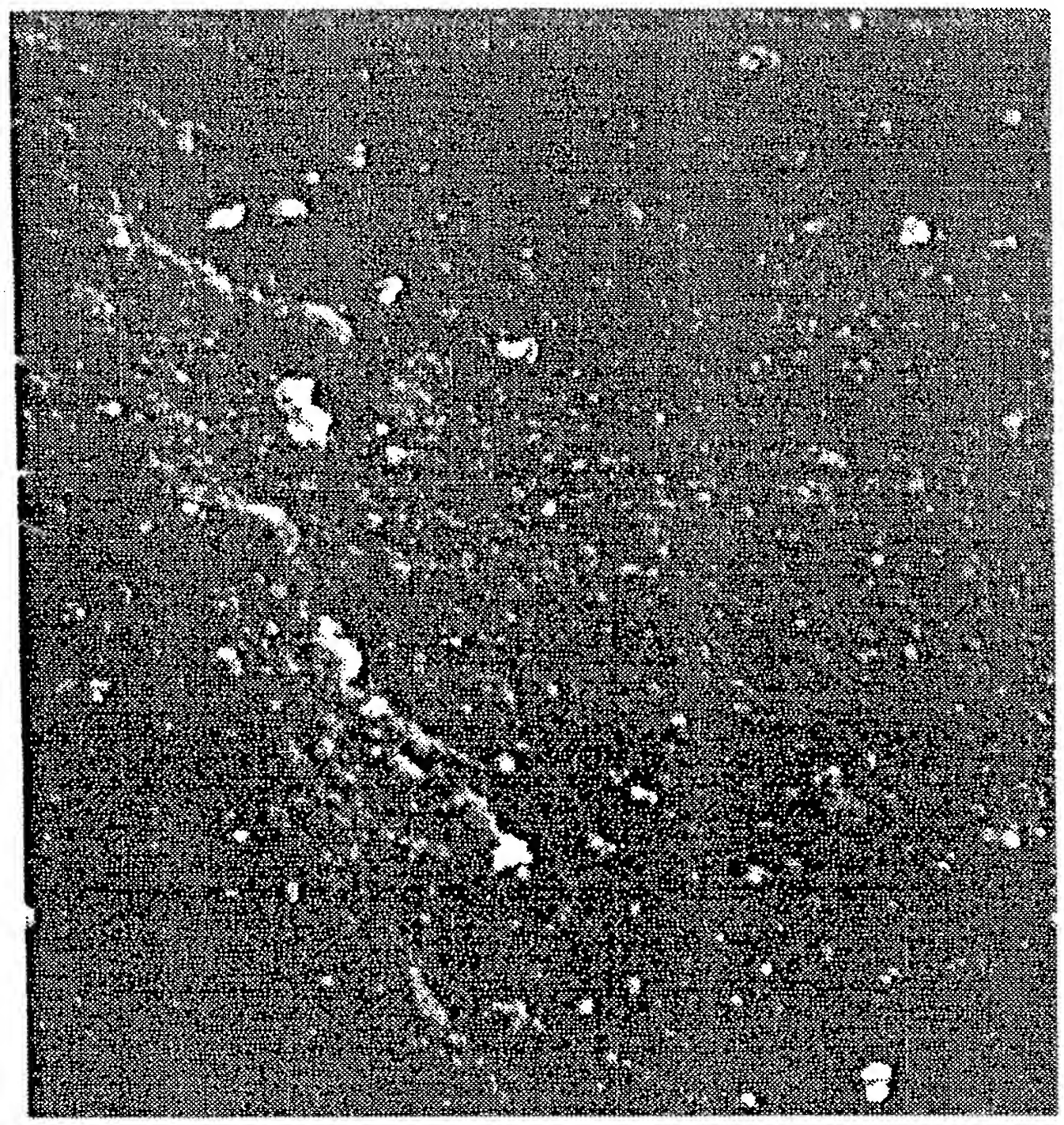

FIG. 3

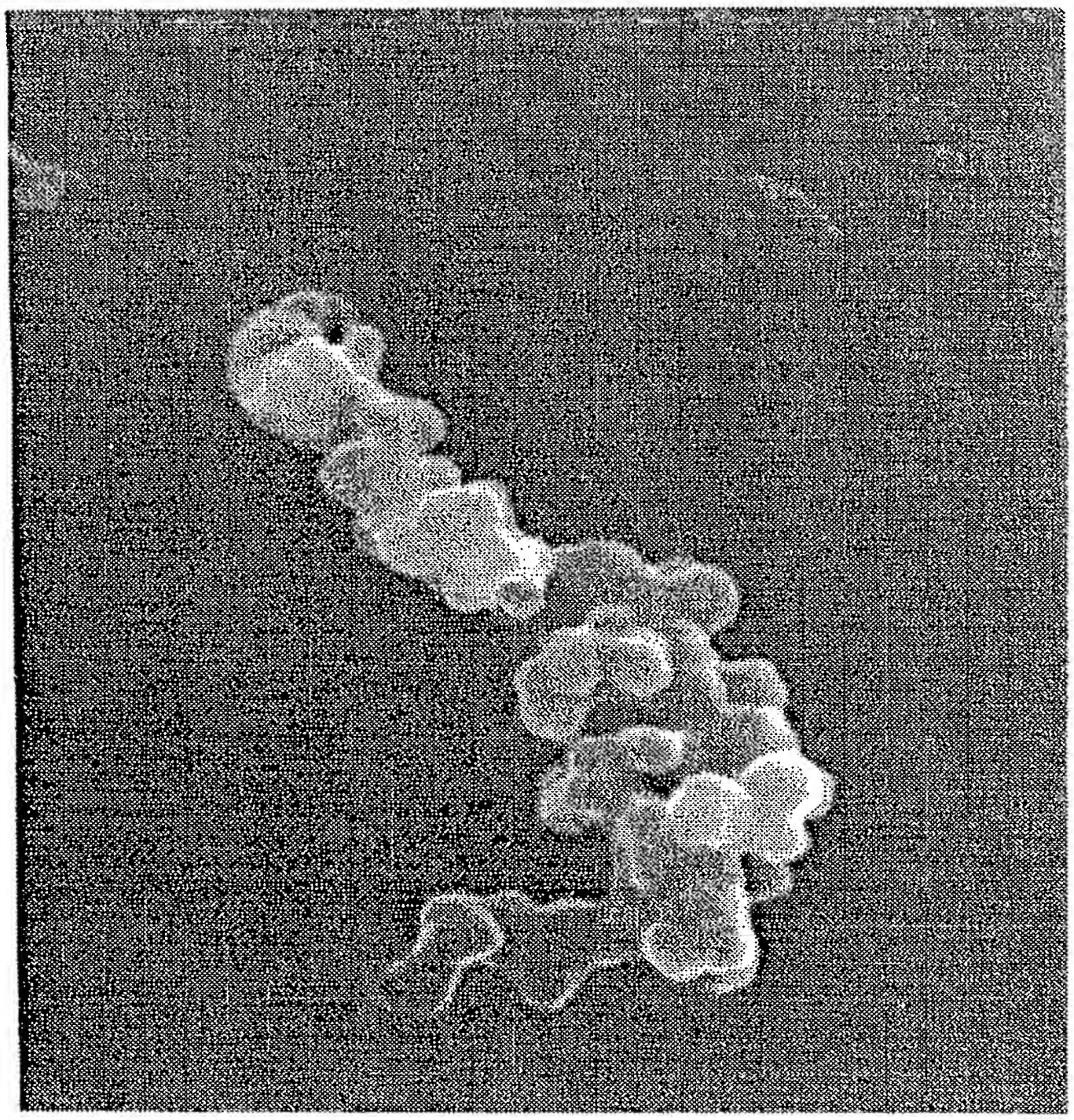

FIG. 4

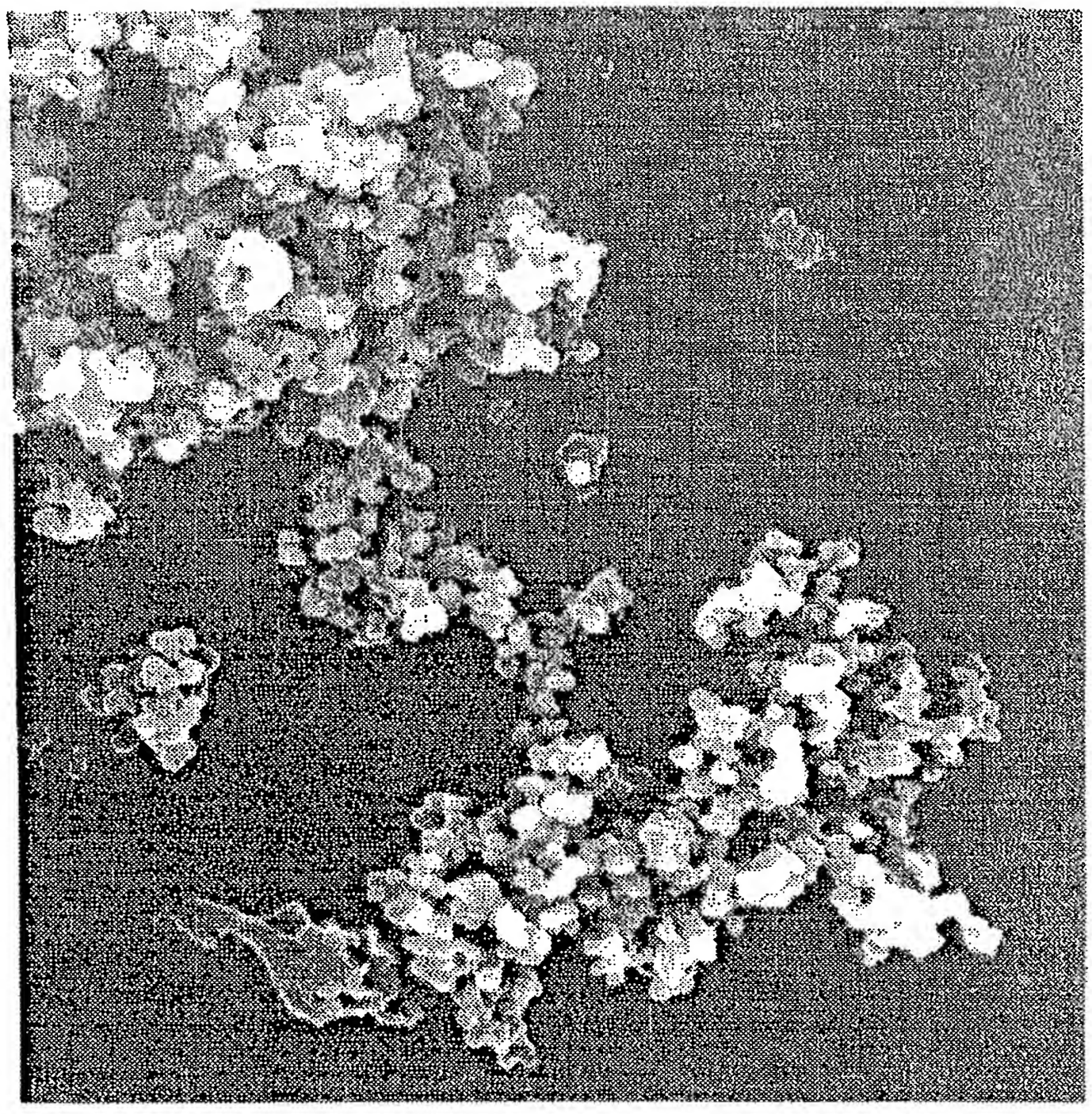

FIG. 5

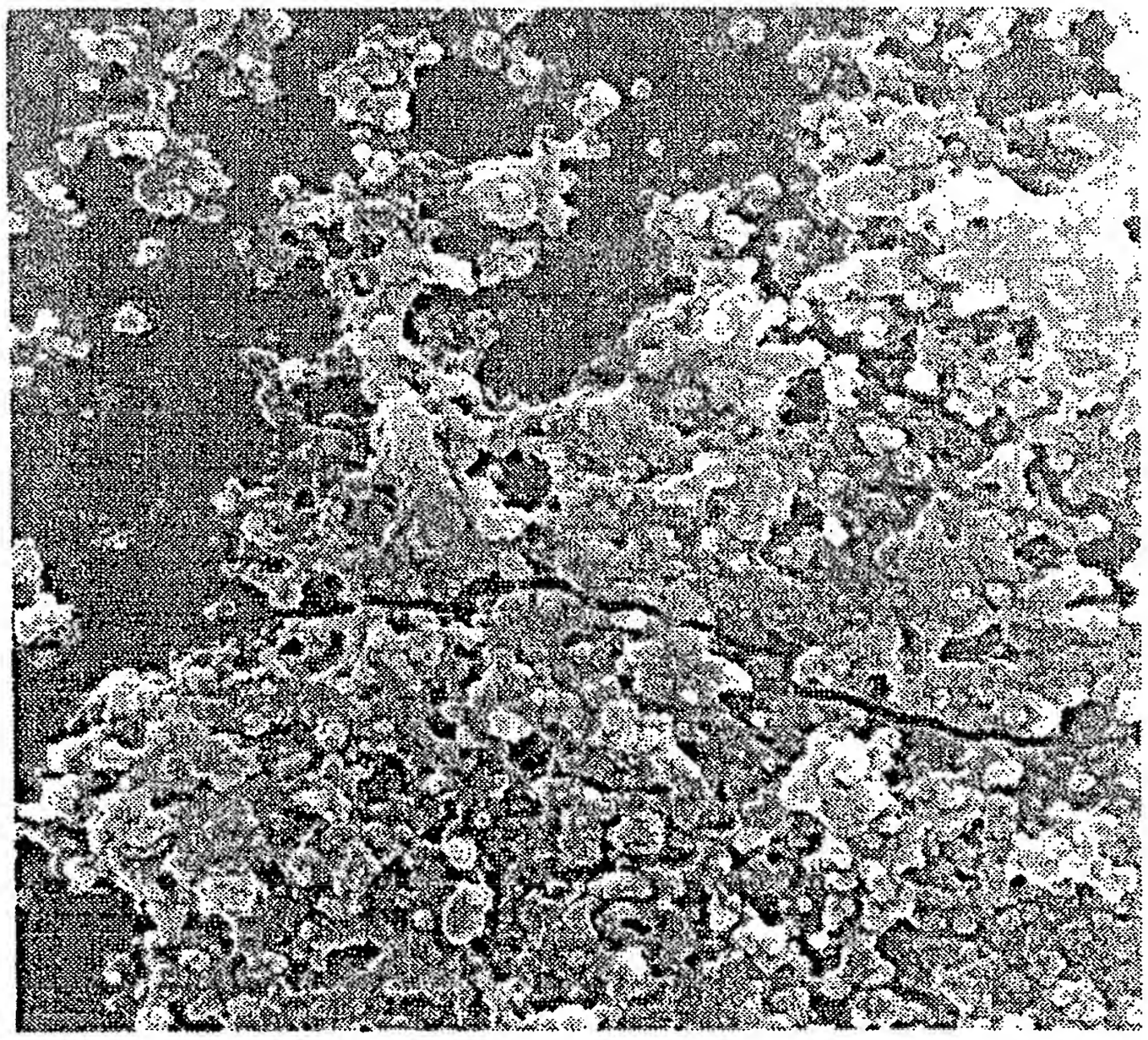

FIG. 6

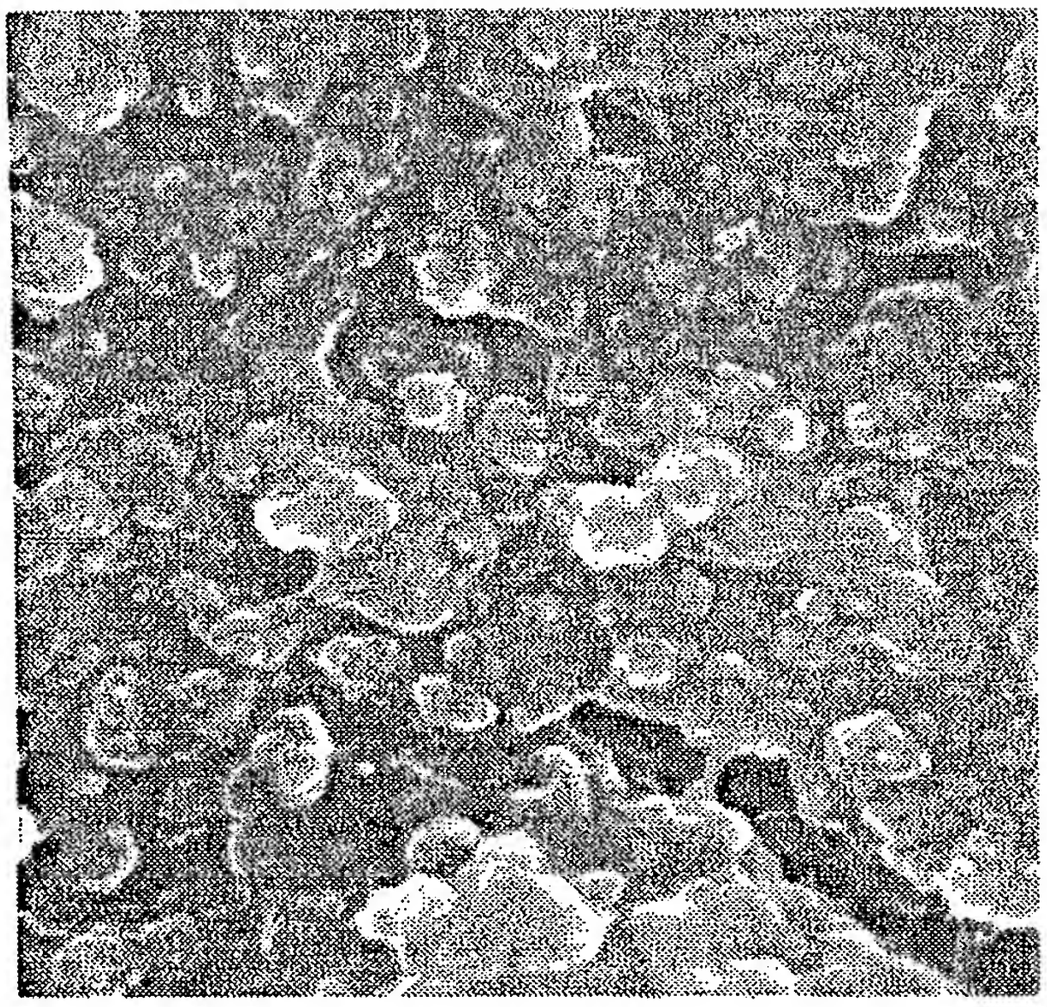

FIG. 7

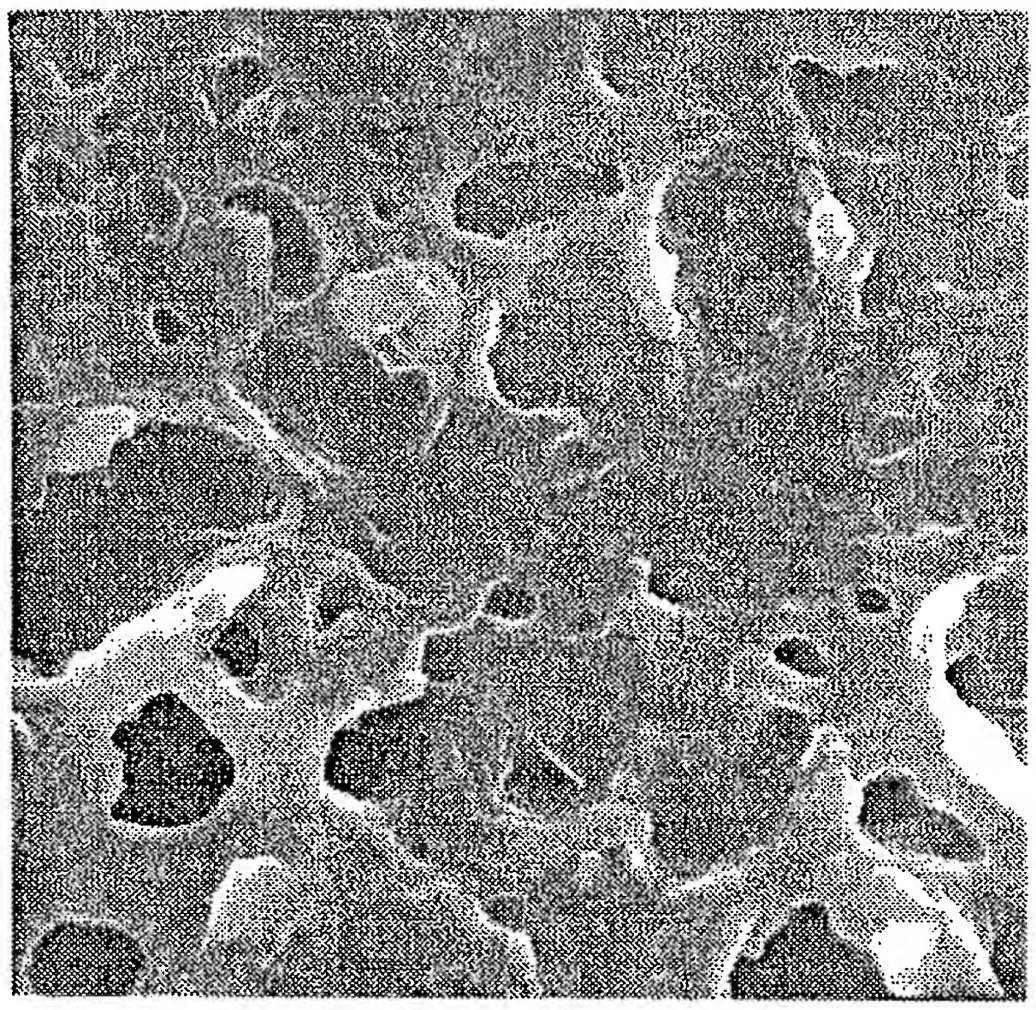

FIG. 8

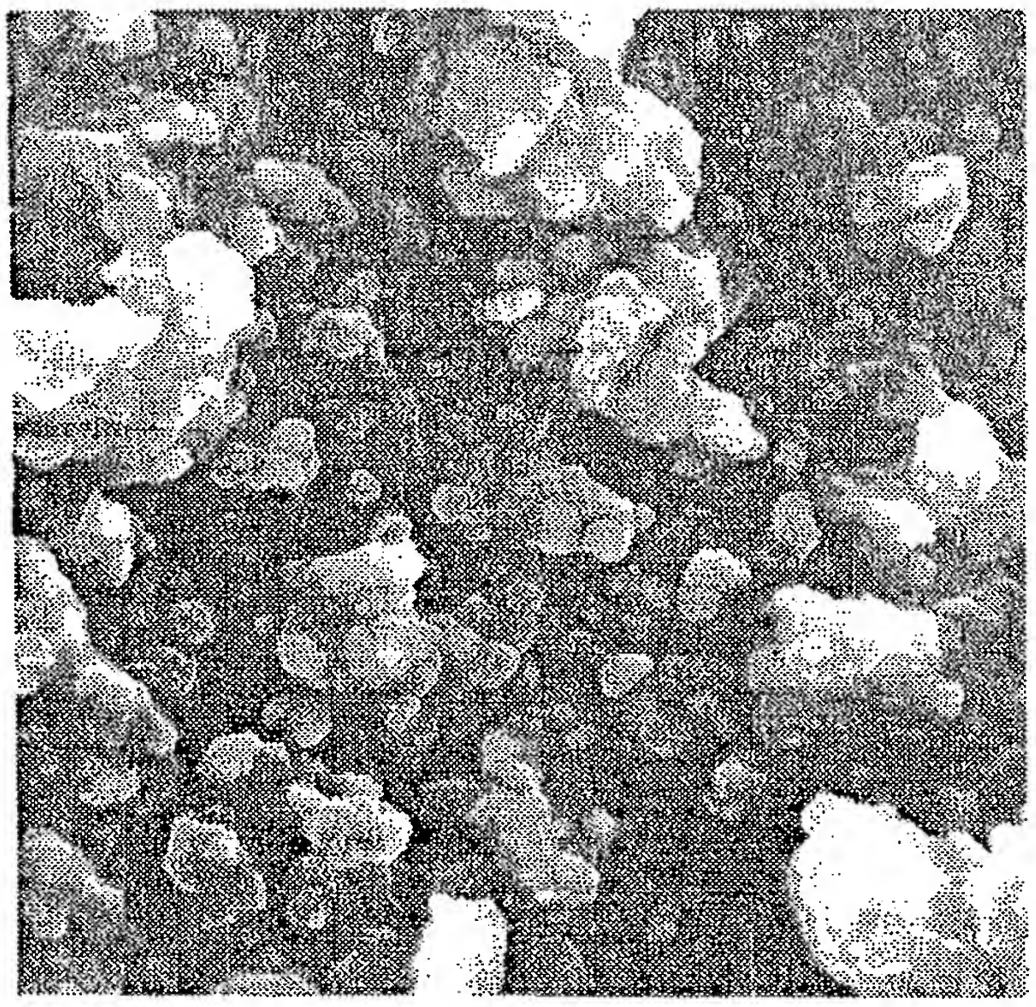

FIG. 9

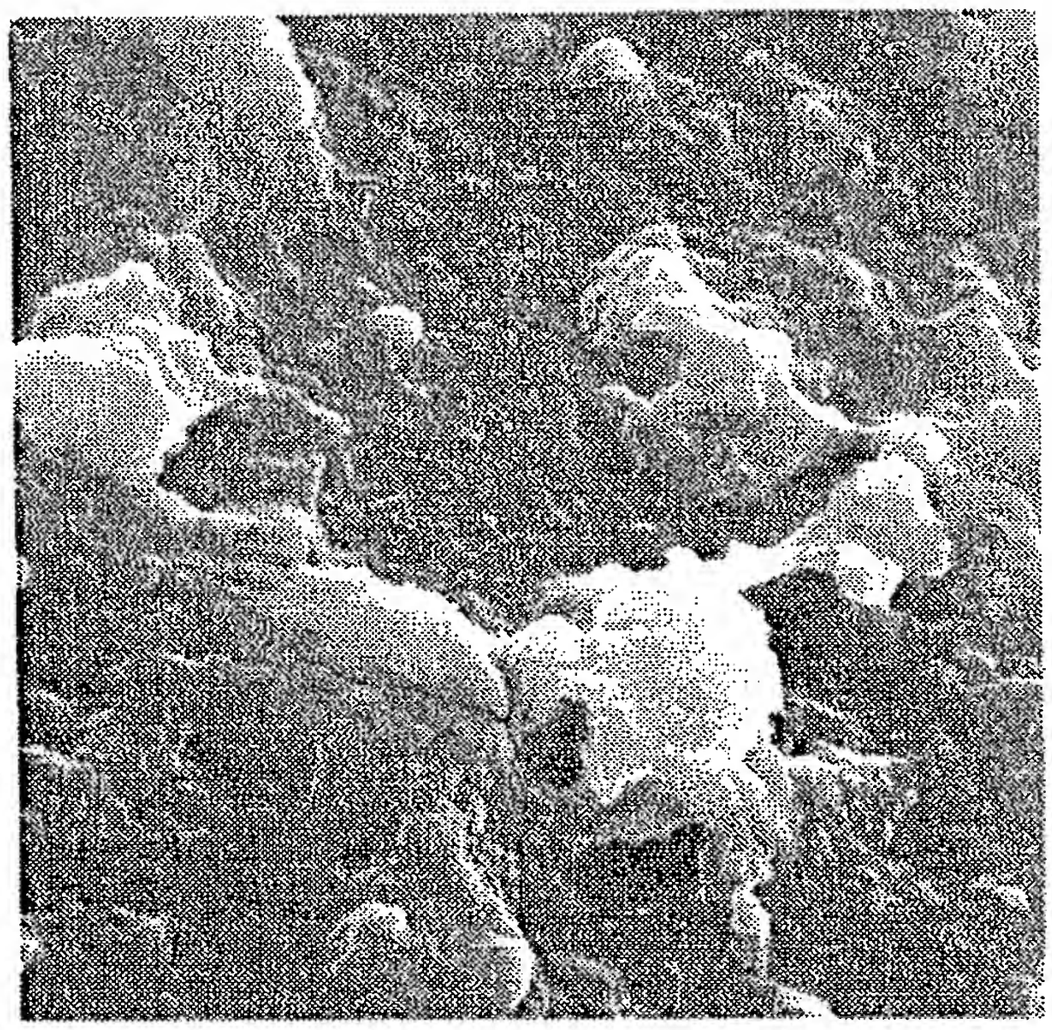

FIG. 10

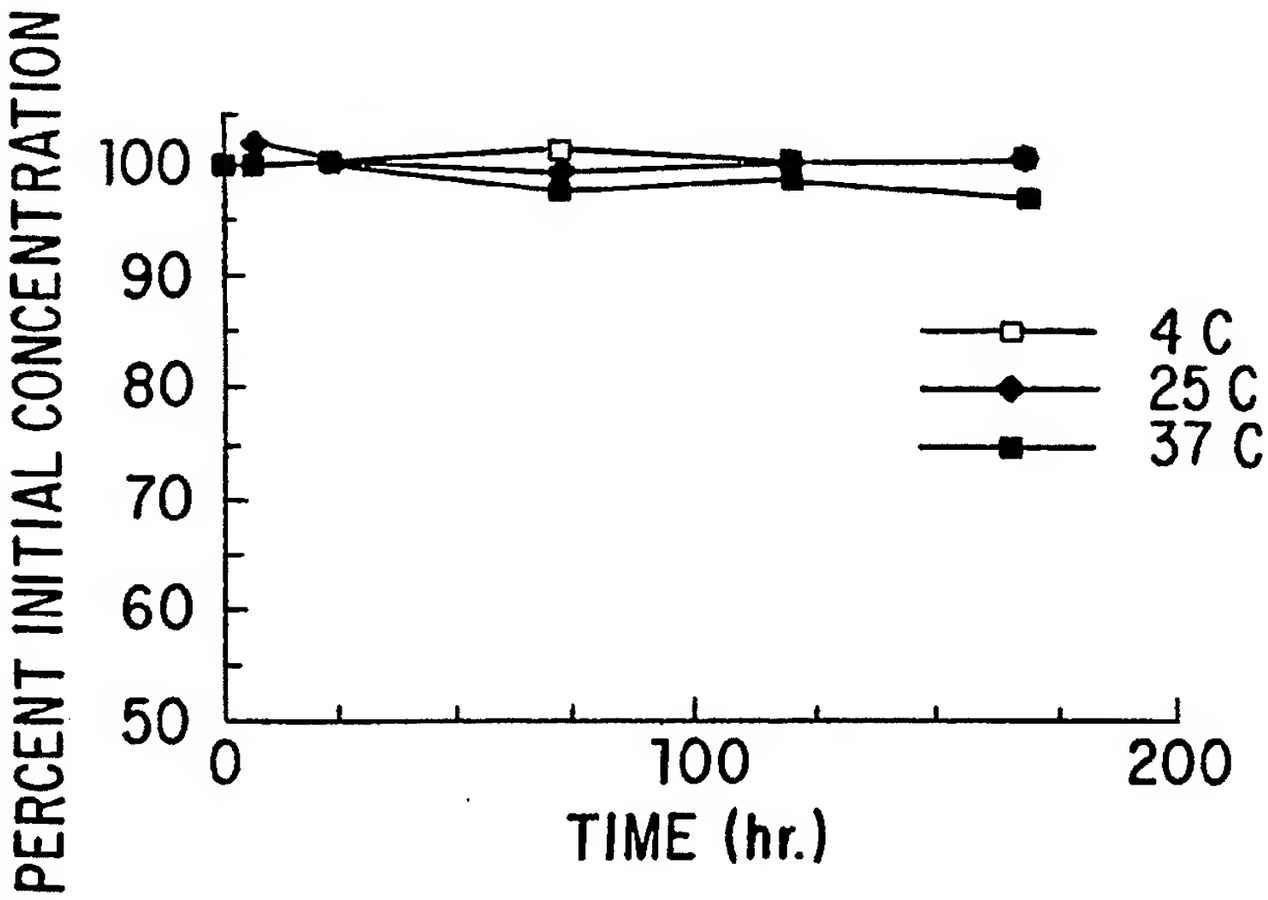

FIG. 11A

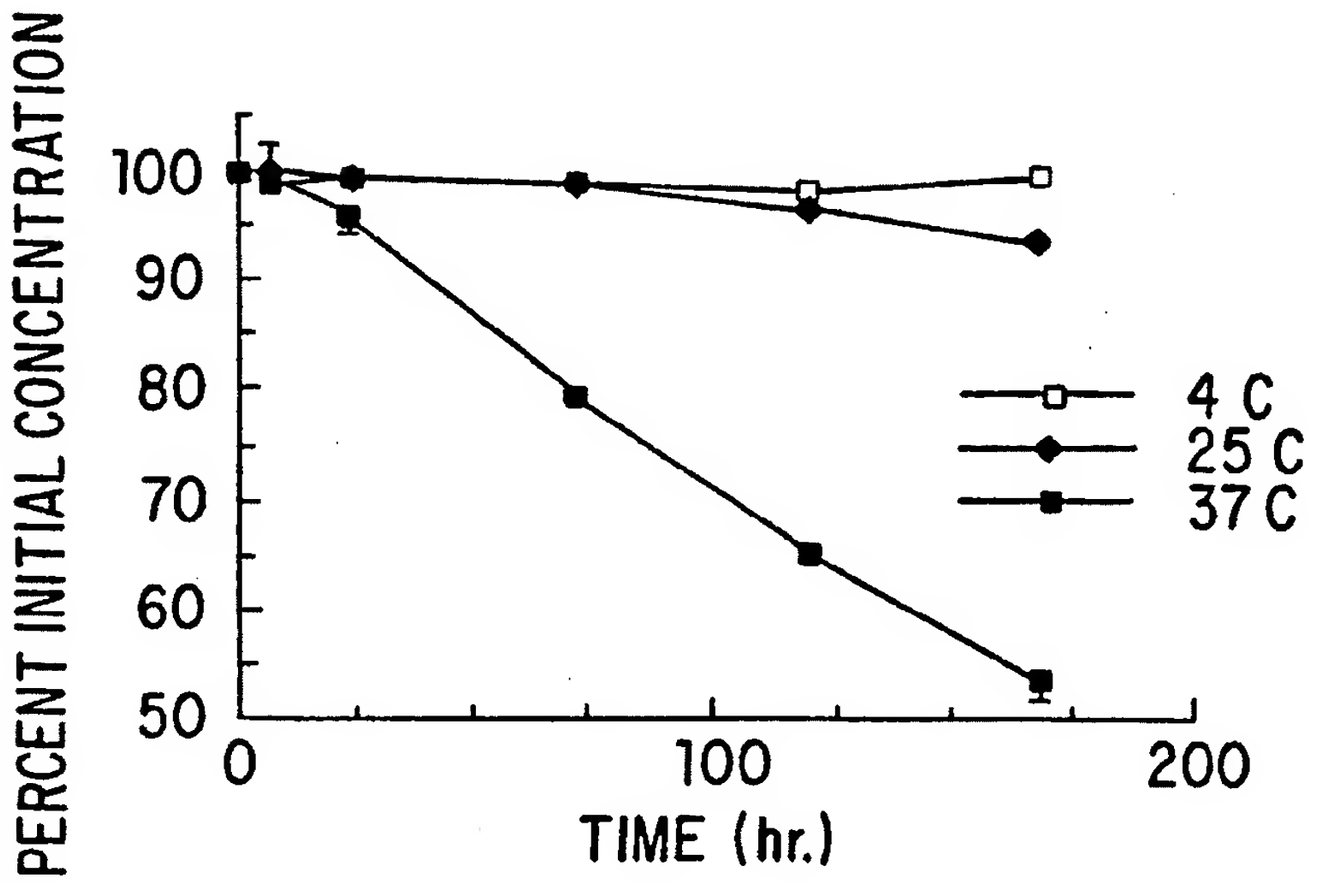

FIG. 11B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10893

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A01N 25/02, 25/08; A61F 2/04; A61M 25/01
US CL :424/405, 409, 423; 604/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/405, 409, 423; 604/53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,Y	US, A, 5,202,449 (HASEGAWA ET AL.) 13 APRIL 1993, column 5, lines 10-13, 29-31.	21-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
20 DECEMBER 1993	16 FEB 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CARLOS AZPURU Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10893

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 13, 14, and 51
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.